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THE PATHOGENESIS OF EXPERIMENTAL HEMOGLOBINURIC NEPHROSIS IN RABBITS, WITH SPECIAL REFERENCE TO THE LATE MANIFESTATIONS *

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The majority of studies on experimental hemoglobinuric nephrosis have placed emphasis on the reproduction of a disease in animals similar to that encountered in man. This has been accomplished with variable degrees of success.[1-6] Most of these studies have been concerned with the early (less than 30 days) phase of hemoglobinuric nephrosis. One study of late (more than 30 days) changes in hemoglobinuric nephrosis has been reported in which partial to complete healing occurred.[6] This, however, was done on only 3 dogs and the material for study was obtained by renal puncture. It is believed, therefore, that additional observations with respect to the late manifestations of experimental hemoglobinuric nephrosis in rabbits by another procedure would be desirable. This has been accomplished by performing a left nephrectomy on those animals which survived acute hemoglobinuric nephrosis. This made possible a comparison of the microscopic findings in the surgically removed kidneys with those obtained at the time of autopsy.

METHOD

This investigation was conducted in conjunction with studies on the relationship of dehydration to the development of hemoglobinuric nephrosis, which have been reported elsewhere.[5,7] Rabbits were deprived of food and water for 3 days. During the next 3 days 7 to 10 intravenous injections of homologous hemoglobin equal to 1.8 gm. per kg. were given to each animal. During this period the rabbits were without food, and the only water they received was administered intravenously (22 to 38

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cc. daily). Under the conditions of this experiment approximately one-third of the rabbits died of hemoglobinuric nephrosis and two-thirds survived after variable elevations of nonprotein nitrogen. A left nephrectomy was performed under nembutal anesthesia 13 to 17 days after the initial injection of hemoglobin in those animals which survived. The operations were done during the period of declining nonprotein nitrogen. Following the nephrectomy, the rabbits were kept from 34 to 116 days and then killed. The tissues were examined and fixed in 10 per cent formalin. The kidneys were divided along the long axis. For microscopic studies, a central longitudinal block of the whole kidney was embedded in paraffin and sections were stained with hematoxylin and eosin. Since pigment casts are located principally in the cortex,[5] this area was selected as the most representative site. The casts in 10 low-power fields were counted and their total number was determined.

Results

Gross Observations

The kidneys which were removed surgically were always congested. This was due to the clamping of the renal vessels prior to the removal of the kidney. The surgically removed kidneys were always larger than one-half the expected average combined weight of 12.5 gm.[8] The surface was smooth in every case. Examination of the surface revealed brown foci measuring 1 to 3 mm. in diameter in 8 of 12 rabbits. On the cut surface, it was possible to distinguish streaks of brown pigment in the cortex in 5 of 12 rabbits.

The kidneys which were removed at autopsy were heavier than those removed surgically in each case (Table I). On the cut surfaces no abnormality other than congestion of the parenchyma was evident.

There was minimal fibrosis of the myocardium in each case due to cardiac puncture for the withdrawal of blood. The lungs and spleens were normal in all rabbits.

Microscopic Findings

The pertinent microscopic changes are tabulated in Table I. The total number of pigment casts observed in 10 low-power fields in the surgically removed kidneys varied from 4 to 188. They were uniformly distributed in the distal convoluted tubules or in sectors as described by Harrison, Bunting, Ordway, and Albrink.[9] In 3 rabbits the accumulation of pigment casts and the tubular dilatation were almost as marked as in some animals which died in uremia. It is of interest that with such extensive pigment precipitation, these rabbits were able to tolerate nephrectomy without developing uremia.

TABLE I

Microscopic Findings in Kidneys Following Intravenous Injections of Homologous Hemoglobin

Rabbit	Sex	Early findings					Late findings				
		Left nephrectomy after hemoglobin	Weight of left kidney	Total* pigment casts	Dilated tubules	Vacuolization of tubular epithelium	Autopsy after surgery	Weight of right kidney	Total* pigment casts	Dilated tubules	Vacuolization of tubular epithelium
		days	*gm.*				*days*	*gm.*			
1	M	16	11.2	21	Moderate	+	34	12.7	4	None	−
2	F	16	7.4	4	Absent	+	34	10.2	0	None	−
3	F	16	11.8	63	Marked	+	34	12.7	0	None	−
4	F	13	9.9	40	Marked	+	47	11.8	0	None	+
5	M	13	10.1	106	Marked	−	47	14.8	5	None	−
6	F	13	8.8	35	Moderate	+	52	11.8	0	None	−
7	M	11	10.1	145	Marked	−	85	11.5	0	None	−
8	F	11	8.2	17	Minimal	+	85	12.8	0	None	−
9	M	15	7.7	40	Moderate	+	89	10.5	0	None	−
10	M	15	11.3	188	Marked	+	89	12.8	1	None	−
11	F	17		48	Moderate	−	105	6.6	1	None	−
12	M	14	8 5	8	Minimal	−	116	15.4	1	None	−

* Sum of pigment casts seen in 10 low-power fields.

There was usually a parallelism between the number of pigmented casts and the degree of tubular dilatation. However, in 2 rabbits (3 and 4) the latter feature was more conspicuous than the former. Associated with tubular dilatation there was flattening of the epithelial cells. Local areas of necrosis of tubular epithelium were not observed in any of the rabbits which survived. It is noteworthy that even in those rabbits which died in uremia such necrosis was minimal and limited to three or four areas in one longitudinal section of the kidney.[5] Vacuolization of epithelial cells in the proximal convoluted tubules and in Henle's loops was present in scattered areas in 8 of 12 rabbits.

Microscopic studies of kidneys removed at autopsy demonstrated the transient nature of hemoglobinuric nephrosis in every instance. In 7 of 12 rabbits no pigment casts were found in the microscopic sections. In 5, the sections contained either isolated or a few pigment casts (Table I). In every case dilatation of tubules was no longer evident.

There was vacuolization of tubular epithelium in only one rabbit. Areas of minimal metastatic calcification of the tubular epithelium were present in 2 animals. There were a few small areas of scattered fibrosis and lymphocytic infiltration in some sections. Since these changes are known to occur spontaneously in rabbits, focal fibrosis with lymphocytic infiltration was not considered significant in this study. Examination of the kidney sections from the early and late phases failed to reveal evidence of tubular atrophy. In 3 rabbits (8, 9, and 10), 85 to 89 days after nephrectomy, there was tubular hypertrophy with regeneration of epithelial cells in the collecting tubules.

Discussion

In previous studies on the production of experimental hemoglobinuric nephrosis, the kidney lesions were always bilateral and of the same degree in both kidneys.[5,7] Therefore it was felt that nephrectomy performed within 13 to 17 days after injecting hemoglobin would serve several purposes. First, it would demonstrate the severity of the changes in the kidneys at the time of removal. Secondly, with knowledge of the microscopic changes, information in respect to functional kidney reserve also could be gained. When the rabbits continued to eat and gain weight after nephrectomy, the function of the remaining kidney was assumed to be adequate. It was highly instructive, therefore, to find 3 rabbits (5, 7, and 10) with moderate to extensive accumulations of pigment casts that were able to survive after the removal of 50 per cent of their functioning renal tissue.

Microscopic comparison of kidney sections removed surgically and at autopsy illustrate the transient nature of hemoglobinuric nephrosis in rabbits when the animals do not die during the acute phase. In each case practically all of the pigment casts disappeared within 34 to 116 days. Significantly enough, these animals were able to achieve complete healing even after the added burden of a left nephrectomy.

This study confirms Flink's [6] observations on the transient nature of experimental hemoglobinuric nephrosis, once the acute phase has passed. I saw no evidence of tubular atrophy as described by Flink. However, regeneration of epithelial cells in the collecting tubules was observed in 3 instances. This apparent discrepancy may be explained by the fact that Flink made repeated punctures to obtain material for biopsy, which undoubtedly injured some nephrons and caused subsequent atrophy of renal tubules.

Summary

When an animal survives the acute phase of hemoglobinuric nephrosis, death does not occur. Under such conditions it must be assumed that

hemoglobinuric nephrosis is a transient disease. The pigment casts, tubular dilatation, and vacuolization of tubular epithelium disappear within 34 to 116 days. Even when there is extensive deposition of pigment, tubular dilatation, and epithelial degeneration, the animals are able to tolerate still further loss of functioning renal tissue in the form of a nephrectomy.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 24

FIG. 1. (Rabbit 7, early.) There was a total of 145 casts in 10 low-power fields, with marked tubular dilatation. The casts and tubular dilatation tended to be diffuse and were limited principally to the cortex. At autopsy 85 days later, examination of the kidney section revealed disappearance of casts and tubular dilatation. Hematoxylin and eosin stain. × 150.

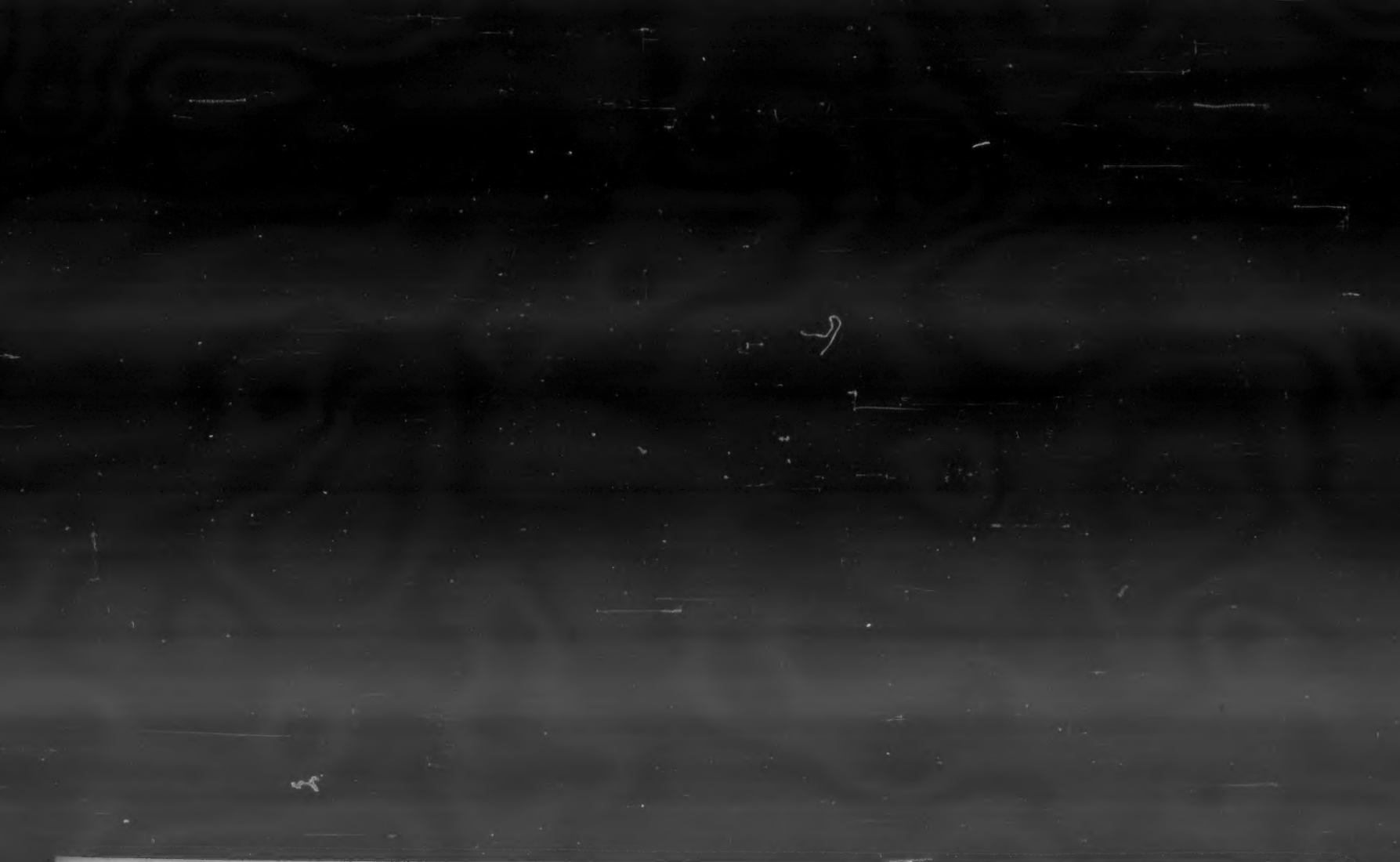
FIG. 2. (Rabbit 5, early.) A total of 106 casts and marked tubular dilatation were found in 10 low-power fields. The involvement tended to be diffuse. The tubular dilatation was more conspicuous than the accumulation of pigment. In the kidney removed at autopsy 47 days later, only a few pigment casts remained and tubular dilatation was absent. Hematoxylin and eosin stain. × 150.

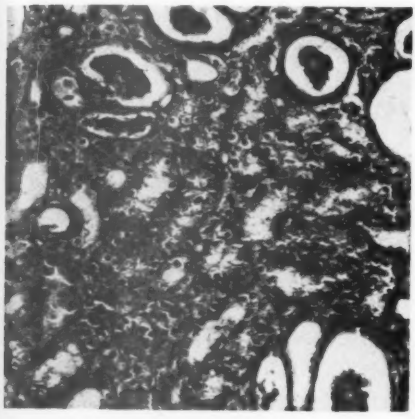
FIG. 3. (Rabbit 3, early.) The pigment casts and tubular dilatation occurred in sectors with uninvolved intervening areas. There was a total of 63 pigment casts in 10 low-power fields with marked tubular dilatation. Hematoxylin and eosin stain. × 150.

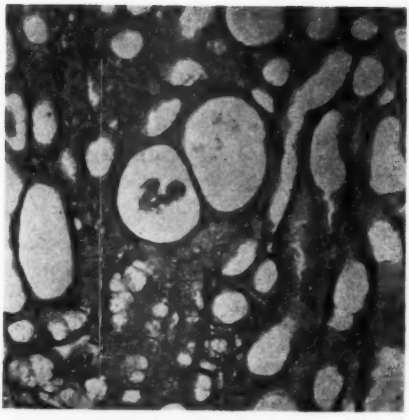
FIG. 4. (Rabbit 3, late.) No casts or tubular dilatation were evident in a section obtained 34 days after surgery. A granular eosin-staining material may be seen in some of the tubules. Hematoxylin and eosin stain. × 150.

FIG. 5. (Rabbit 10, early.) This rabbit had a total of 188 pigment casts with marked tubular dilatation in 10 low-power fields. The involvement tended to be diffuse. Hematoxylin and eosin stain. × 150.

FIG. 6. (Rabbit 10, late.) Eighty-eight days following nephrectomy, one pigment cast was observed in 10 low-power fields. There was then no evidence of tubular dilatation. Hematoxylin and eosin stain. × 150.







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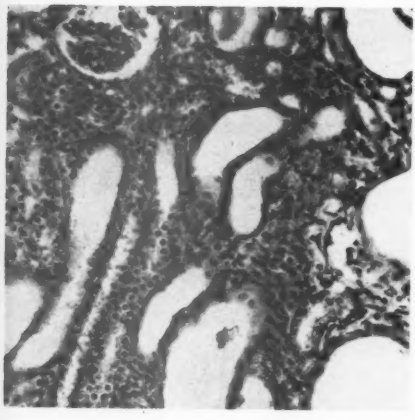

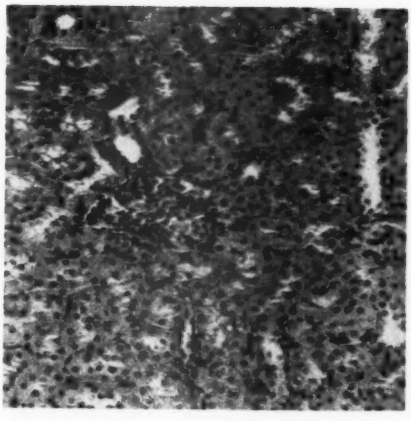

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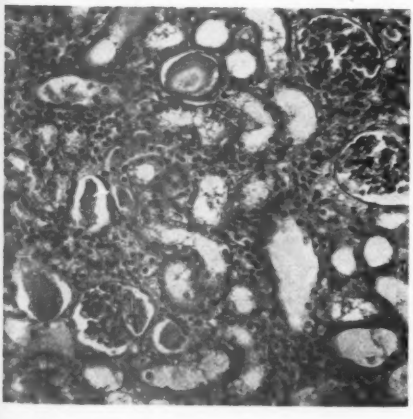

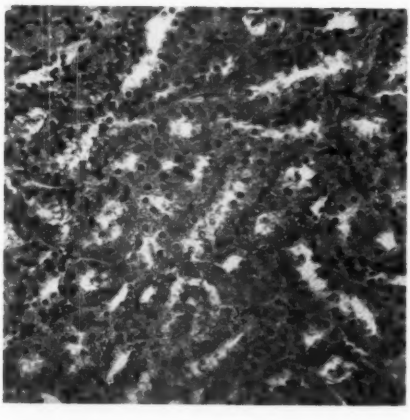

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Lalich Experimental Hemoglobinuric Nephrosis

HEMOGLOBINURIA (BLACKWATER FEVER) IN MONKEYS
A Consideration of the Disease in Man *

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Although it was observed as early as 1932 that hemoglobinuria occurred in *Macacus rhesus* monkeys infected with *Plasmodium knowlesi*, apparently the mechanism by which it develops has not been established.[1,2] Furthermore, even with this experimental animal, the pathogenesis of blackwater fever in man has not been explained satisfactorily. Recent experimental studies on hemoglobinuria [3-7] and observations on acute malarial infections in ducks [8-11] and monkeys [12] have served as a stimulus to consider the problems of the mechanism of the development of hemoglobinuria in monkeys with malaria, and to consider the pathogenesis of the corresponding process in man.

In the original observations with *P. knowlesi* it was emphasized that hemoglobinuria frequently occurred when there was a severe infection.[1] The prerequisite, therefore, for the experimental production of hemoglobinuria is a severe infection with *P. knowlesi* in a *M. rhesus* monkey. The mortality rate is very high in this host with this plasmodium.[1,2] All animals do not develop hemoglobinuria; however, it frequently occurs terminally. The renal lesions accompanying a severe malarial infection in the monkey have been described.[1,2,12] There is general agreement in those studies that degeneration occurs in the epithelial cells lining the convoluted portion of the renal tubules, and there are occlusions within the lumina of the collecting tubules.

The recorded studies on blackwater fever in man are numerous. French physicians from 1851 to 1859 were among the first to call attention to the occurrence of dark urine in patients with malaria, although the disease had been known since the days of Hippocrates.[13] Thomson [13] considered the late recognition of this complication of malaria as due to several factors, "the most important being the exploration and development of the tropics by non-immune white races, and finally the settlement of the non-immunes in these areas for the development of trade and agriculture."

Blackwater fever is the term used to designate the occurrence of hemoglobin pigments in the urines of patients infected with malarial parasites. Hemoglobinuria may occur in a variety of conditions unasso-

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ciated with malaria.[14-17] It is more often associated with the more virulent forms of the disease in man, that is, with *P. falciparum* infections. This study is based upon clinical and pathologic observations made upon 10 *M. rhesus* monkeys infected with *P. knowlesi.*

Materials and Methods

M. rhesus monkeys were inoculated with *P. knowlesi.* The size and apparently the age varied widely in this group of 27 animals. Ten developed hemoglobinuria (Table I). The infection was transferred directly from donor monkeys, by intraperitoneal, subcutaneous, or intravenous inoculation of parasitized blood. Five monkeys (7, 177, 131, 29, and 124), were autopsied immediately following death. The presence of hemoglobinuria was determined by observing the passage of dark urine and by examining the urine obtained from the bladder at the time of autopsy. Blood for hematologic studies was obtained, usually from the lobe of the ear. However, it was necessary to obtain it from branches of the saphenous veins and the heart in moribund animals. Standard technics were used for the red blood cell counts. Blood smears were treated with a combination of Wright's and Giemsa's stains. The parasitemia was determined by counting the number of parasitized cells per 1000 red blood cells. The method used for the determination of carbon dioxide was that outlined by Peters and Van Slyke [18] for the volumetric blood gas apparatus.

The tissue used for histologic studies was fixed in Bouin's fluid. Paraffin sections were prepared and stained by the following methods: Hematoxylin and eosin, hematoxylin and phloxine, Mallory's azocarmine, and the Prussian blue stain for iron.

Experimental Findings

All of the monkeys, as shown in Table I, had a severe infection preceding the time of death except monkeys 227 and 12. The former was killed following a period of hemoglobinuria and the disappearance of parasites from the peripheral blood, and the latter had 300 parasitized cells per 1000 red blood cells 4 days preceding death. In a majority of these monkeys there was a marked anemia and a high degree of parasitemia at the time of the appearance of hemoglobinuria. The urine from monkey 131 was reddish brown; innumerable red blood cells were present, some being in small groups, while others formed casts. Many epithelial cells were present. The urine from monkey 7 was reddish and contained a large amount of fine, granular débris and a few epithelial cells. No red cells were observed in an uncentrifuged specimen. The

TABLE I

Data on 10 Monkeys in Which Hemoglobinuria Occurred Following Inoculation with P. knowlesi

Number	Interval between inoculation and death	When hemoglobinuria occurred			Treatment	Remarks
		Interval before death	Red blood cell count	Parasitized red blood cells per 1000		
			millions			
131	10 days	0	1.38	324	Quinine, oxygen	
29	6 days	24 hrs.	2.06	407	Quinine, oxygen, blood	Hemoglobinuria occurred before treatment began
177	6 days	30 hrs.	2.08	346	Quinine	
227	19 days	6 days	3.06	95	Quinine, blood	Hemoglobinuria stopped following second injection of quinine
7	5 days	2 hrs.	0.95	536	None	
10	5 days	14 hrs.	5.01	230	Quinine, oxygen, blood	Red blood cell count and parasite count made approximately 38 hrs. before death; hemoglobinuria occurred before quinine was given
12	7 days	24 hrs.	1.17	84	Quinine, oxygen	
14	6 days	26 hrs.	4.01	584	Quinine, blood	Hemoglobinuria at time quinine was given
124	8 days	2 hrs.	2.67	695	Quinine, oxygen, blood	Red blood cell count made 9 hrs. before death; parasite count, 3 hrs.
151	6 days	24 hrs.	2.31	688	Oxygen	Hemoglobinuria occurred before oxygen was given

urine from the bladder of monkey 29 appeared normal. However, 24 hours had elapsed since hemoglobinuria was observed in this animal.

There is no evidence in this study to indicate that hemoglobinuria results from the administration of blood, oxygen, or quinine. Monkey 7 received none, while monkeys 10, 14, and 29 were voiding dark urine at the time quinine was first given. Monkey 151 was given oxygen following the appearance of bloody urine. Monkey 227 developed hemoglobinuria 12 days following the injection of blood, and at this time he also had malaria. The urine from this monkey appeared normal following the second injection of quinine and remained grossly normal for 4 days, during which time three more injections of quinine were administered.

Monkey 7 illustrates the rapidity with which malaria may develop, and also demonstrates the severity of the anemia that may accompany it. This monkey had a severe acidosis at the time of death, as shown below.

Inoculated with P. knowlesi

Days	Red blood cell count *millions*	Parasitized cells per 1000 red blood cells
1	5.89	0
2	5.66	0
3	5.67	18
5 8:15 a.m.	1.57	803
5 12:00 noon	0.95	536
5 1:30 p.m.	Moribund, voiding bloody urine.	
	Killed by bleeding from heart.	
	CO_2 content of plasma, 11.1 volumes per cent.	
	CO_2-combining power of plasma, 9.0 volumes per cent.	
	Hematocrit, 0.7 per cent.	

Macroscopically, the spleen and liver in every monkey were enlarged and deeply pigmented. In fact, all tissues were pigmented. Since the pathologic changes in monkeys infected with *P. knowlesi* have been reported previously,[12] only the renal lesions are described here. Usually the kidneys were enlarged and deeply pigmented, and fluid exuded from the cut surface. In contrast to these changes in the kidneys of monkeys showing hemoglobinuria at the time of death, the kidneys from monkey 227, the animal that recovered from hemoglobinuria, were normal in size and color and the cut surfaces were dry. No casts were present in the kidneys of this monkey.

The glomeruli in all of the monkeys showed no significant changes. The epithelial cells lining the convoluted portion of the renal tubules always were injured. Sometimes they were so swollen that the tubular

lumina were essentially occluded (Figs. 2 and 3). In other instances, these cells were fragmented and sometimes they were desquamated into the lumina of the tubules. In the cytoplasm of many of these cells there were small masses of yellowish brown pigment, some of which stained faintly blue with the Prussian blue method for hemosiderin (Fig. 2). Larger amounts of a similar appearing pigment were present in the lumina of the renal tubules. The epithelial cells lining the loops of Henle and the collecting portions of the tubules sometimes were desquamated. This change was observed more frequently in the vicinity of large casts. The type of cast and its location within the tubules varied (Figs. 4 to 7). Some were large, homogeneous, pink-staining casts, others were formed by groups of deep-eosin-staining granules approximately the size of a red cell, while others were formed by a much smaller but similar staining granule (Fig. 5). Desquamated epithelial cells were conspicuous in some of the tubules. Amorphous and yellowish brown material also was present in the lumina of the tubules. Often a combination of the above constituents formed the casts. A large number of casts were located in the collecting portions of the tubules (Fig. 7). However, in some of the monkeys many casts were present in the convoluted portions (Fig. 5). No significant lesions were noted within the interstitial tissue. The lumina of the renal blood vessels was filled with parasitized red cells. No specific vascular lesions were noted.

Discussion

Observations in this study show that hemoglobinuria occurs in *M. rhesus* monkeys infected with *P. knowlesi*. It is interesting to note that the younger monkeys with a severe infection more often show this complication. The time of occurrence of the hemoglobinuria is influenced by the degree of parasitemia. In each instance there is a high degree of parasitemia, usually accompanied by a severe anemia. Thomson,[13] in studying blackwater fever in man, has emphasized the rôle of anemia. He observed "that pre-blackwater fever cases in every instance seen before the attack showed evidence of anaemia very similar in appearance to that of pernicious anaemia .... It is obvious that the malarial anaemia before the onset of blackwater must be an important factor in the severity of the symptoms following a haemolysis, because, if the hemoglobinuria is severe in a case whose blood has been previously depleted of haemoglobin, the prognosis will be a very bad one .... The degree of anaemia in fatal cases of blackwater is often intense and rapidly progressive."

Observations on the carbon dioxide-combining power of the plasma

were made in only one monkey. It is interesting, however, to find that in this monkey a severe terminal acidosis * was present at the time of the hemoglobinuria. Fairley and Bromfield [19] observed in monkeys infected with *P. knowlesi* "the development of typical continuous acidotic dyspnoea associated with similar biochemical findings." It has been observed in ducks infected with *P. lophurae* that an increase in the carbon dioxide content of the plasma occurs with an increase in the degree of the parasitemia and the anemia.[20] Observations on the blood of patients with malaria for the presence of either acidosis or alkalosis apparently have been made infrequently; however, the studies of Fairley and Bromfield showed an acidosis. Thomson [13] found the urine to be acid in nearly every instance of blackwater fever; Burkitt,[21] likewise, found it acid.

At autopsy all of the monkeys had enlarged livers and spleens and the tissues were deeply pigmented. The reticulo-endothelial system was filled with pigment.[12] The lesions in the kidney were primarily tubular. The cells lining the convoluted portions of the tubules were severely injured, some were swollen and granular, others vacuolated, while some cells were absent, either as the result of complete degeneration or because of desquamation. No significant changes were observed in the epithelial cells either in the loops of Henle or in the collecting portion of the tubules. There were cellular débris, epithelial cells, granules of hemoglobin and hemoglobin casts within the lumina of the tubules. Apparently, the histologic changes in the kidneys of these monkeys are identical with those observed by Flink [22] in dogs that developed hemoglobinuria following the intravenous injection of hemoglobin. The quantity of materials within the lumina of the renal tubules in these monkeys appears to be insufficient to produce anuria in the manner suggested for cases of the so-called "lower nephron nephrosis."[23] Certainly, casts in the lumina of tubules may produce anuria; however, anoxia and shock appear to contribute more to the occurrence of anuria in monkeys with hemoglobinuria accompanying malaria. The dogs in Flink's experiments did not develop anuria, although there were innumerable casts in the tubules.[22] From the study of a case of post-transfusion uremia, Ayer and Gauld [24] concluded that "There is no evidence that the casts by any strictly mechanical obstruction produce either the oliguria or the epithelial necrosis." Tubular cell injury is the predominant lesion in some transfusion reactions.

* Since the carbon dioxide-combining power was 9 volumes per cent and carbon dioxide content 11.1 volumes per cent, the alveolar carbon dioxide tension must have been abnormally high and, therefore, blood pH was probably decreased. This would represent a true acidosis.

In *P. knowlesi* infections there occurs a tremendous destruction of red blood cells by the plasmodia. A majority of this hemoglobin is converted into hematin by the parasites, and ultimately it is phagocytized by the cells of the reticulo-endothelial system. Varying amounts of hemoglobin-containing pigments from the parasitized cells are liberated either at the time the cell ruptures or following its phagocytosis. Such free pigments are removed from the blood stream by the reticulo-endothelial cells and are utilized by the host.[25] Hemoglobinemia has been observed by different investigators in malaria.[26-28] Yorke, Murgatroyd, and Owen[26] stated that "if we correlate these observations on the haemoglobinaemia and those on the red cell count and degree of haemoglobinuria, we find that a distinct parallelism exists. At the period of most intense hemolysis —as judged by the fall in the red cell count and the concentration and amount of haemoglobin in the urine—we find the highest degree of haemoglobinaemia." Thompson[13] stated "that haemoglobinemia precedes haemoglobinuria, and that in those cases where it cannot be detected it has been missed owing to its rapid elimination from the serum." Usually when hemoglobinuria occurs, there is a history of multiple attacks of malaria; however, patients may develop dark urine with their first attack of virulent malaria.[13] Flink,[22] in discussing hemoglobinuria, stated: "There is some evidence from clinical cases to support the thesis that the plasma hemoglobin level and/or the amount of hemoglobin liberated are the important factors." Bordley[29] reviewed 15 cases of transfusion reactions and found that the 10 patients who died received an average of 564 cc. of blood, while those that recovered received an average of only 314 cc. No patient receiving less than 350 cc. died, and none receiving more than 540 cc. recovered. Apparently there is a wide variation in the amount of hemoglobin pigments in the plasma of patients with blackwater fever.[26,27,30]

Yuile's[7] comment in 1942 on hemoglobinuria certainly seems applicable to malaria: "The literature contains a group of conflicting theories, each of which tends to be documented in a self-perpetuating fashion and few attempts have been made to explain the phenomenon of hemoglobinuria in the light of facts uncovered in other fields of renal research." Hemoglobin, when injected intravenously, is eliminated through the glomerulus and the rate is directly proportional to the concentration in the plasma.[4] Any increase in plasma concentration above the level at which maximum reabsorption occurs in the convoluted tubules will cause the hemoglobin to appear in the urine.[4] Fairley[5] believed that extracellular circulating hemoglobin is treated as a foreign substance which disintegrates, and the body eliminates it as soon as

possible by three major routes: (a) Absorption by the reticulo-endothelial system; (b) intravascular catabolism of hemoglobin; and, (c) by renal excretion.

Apparently there is no basic reason why extracellular hemoglobin pigments associated with a malarial infection would be eliminated in a fashion different from that which follows an intravenous injection of hemoglobin in dogs and, presumably, other mammals. The granules present in the cytoplasm of the epithelial cells of the renal tubules of the monkeys with hemoglobinuria were similar to those observed in the dog [3,4] following the intravenous injection of hemoglobin and in man following a transfusion reaction.[29] This process of storage and disintegration of the hemoglobin molecule is not unlike that seen in the liver, spleen, and lymph nodes.[7] Degeneration of the tubular epithelial cells, as observed in the monkeys in this study, may be the result of acidosis. Apparently, tubular lesions may occur following the intravenous injection of large amounts of hemoglobin in normal animals. Anderson and Morrison [31] observed tubular lesions following the intravenous injection of hematin in normal monkeys. In summarizing the pathologic findings associated with blackwater fever, Thomson [13] said: "These changes are what we would expect in an acidosis, and, according to Professor Bartlett, were exactly of the degree and type one gets with severe acidosis from other causes."

The results observed by Bing [32] in acidotic dogs injected intravenously with hemoglobin pigments apparently are identical with the changes observed in these malaria-infected monkeys with hemoglobinuria. Bing found that "The intravenous infusion of crystalline methemoglobin into dogs rendered acidotic with ammonium chloride is followed by a fall in the effective renal plasma flow, glomerular filtration rate, and the tubular reabsorptive capacity for glucose. The renal lesion in acidotic dogs infused with methemoglobin consists of hydropic degeneration of the proximal convoluted tubules, cellular necroses in the distal segment and plugging of the collecting tubules with hyaline and in some instances with pigmented casts. Dilatation of the collecting tubules and glomerular damage are absent."

The presence or absence of either oliguria or anuria was not determined in these monkeys. However, each frequently occurs in blackwater fever in man. It is significant to remember that in severe malarial infections, both in man and experimental animals, symptoms of shock may occur.[12,33-35] Glomerular filtration is influenced by the blood pressure, and in the presence of shock glomerular filtration is significantly decreased. Van Slyke and his group [36] demonstrated that a severe re-

duction occurred in the renal blood flow during circulatory failure. This was often followed by anuria and death of the animal. It is suggested that such a mechanism is more significant in producing anuria in blackwater fever than obstruction in the lumina of the tubules.

Summary

It appears that hemoglobinuria in *Macacus rhesus* monkeys infected with *Plasmodium knowlesi* and blackwater fever in man accompanying malarial infections are similar in their pathogenesis. In both there is an excess of hemoglobin pigments in the plasma resulting from either a high degree of parasitemia or a complete filling of the reticulo-endothelial cells with pigments and their inability to remove additional pigment from the blood. The circulating pigments are filtered through the glomeruli and some are absorbed by the tubular epithelial cells. Because of the presence of an excessive quantity of these pigments in the glomerular filtrate, and because the epithelial cells in the convoluted portions of the tubules are injured by the acidosis, much of the pigment that enters the tubules is eliminated through the urine. In the presence of acid urine many hemoglobin casts form within the lumina of the tubules. A varying number of epithelial cells may also be found in the lumina of the tubules, resulting from the cellular injury accompanying acidosis.

The pathologic changes in the renal epithelial cells of monkeys infected with *P. knowlesi* are similar in animals that do, and in those that do not, show hemoglobinuria. This would indicate that the lesions in the tubules do not result from the hemoglobinuria. The results are essentially the same in acidotic dogs injected with hemoglobin, in humans with a virulent malarial infection and showing hemoglobinuria, and in monkeys with a severe *P. knowlesi* infection and passing black-colored urine. This would indicate that the basic principles recently established in regard to the excretion of hemoglobin pigments by the kidney are applicable to the problem of blackwater fever in man.

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[*Illustrations follow*]

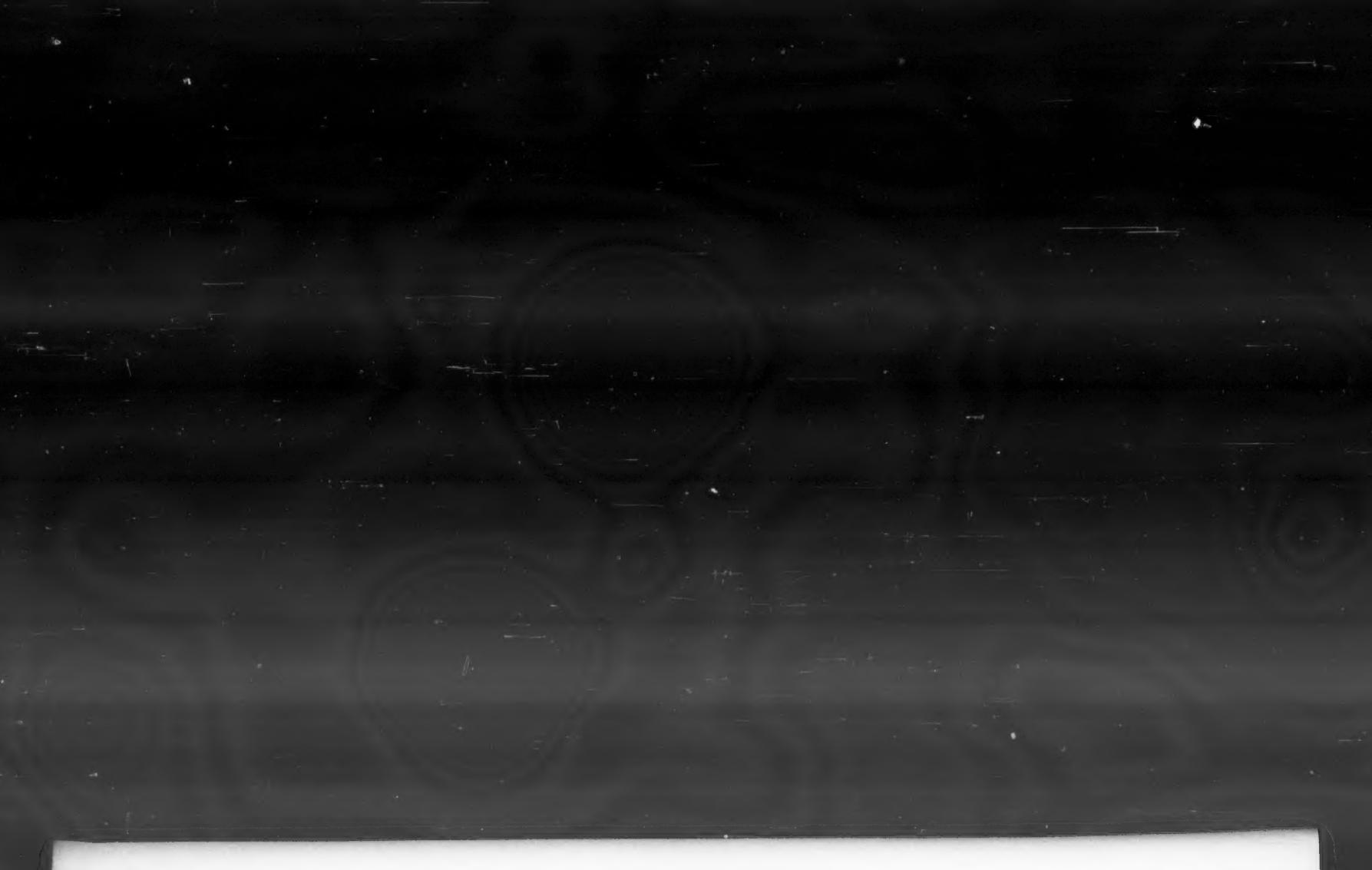
DESCRIPTION OF PLATES

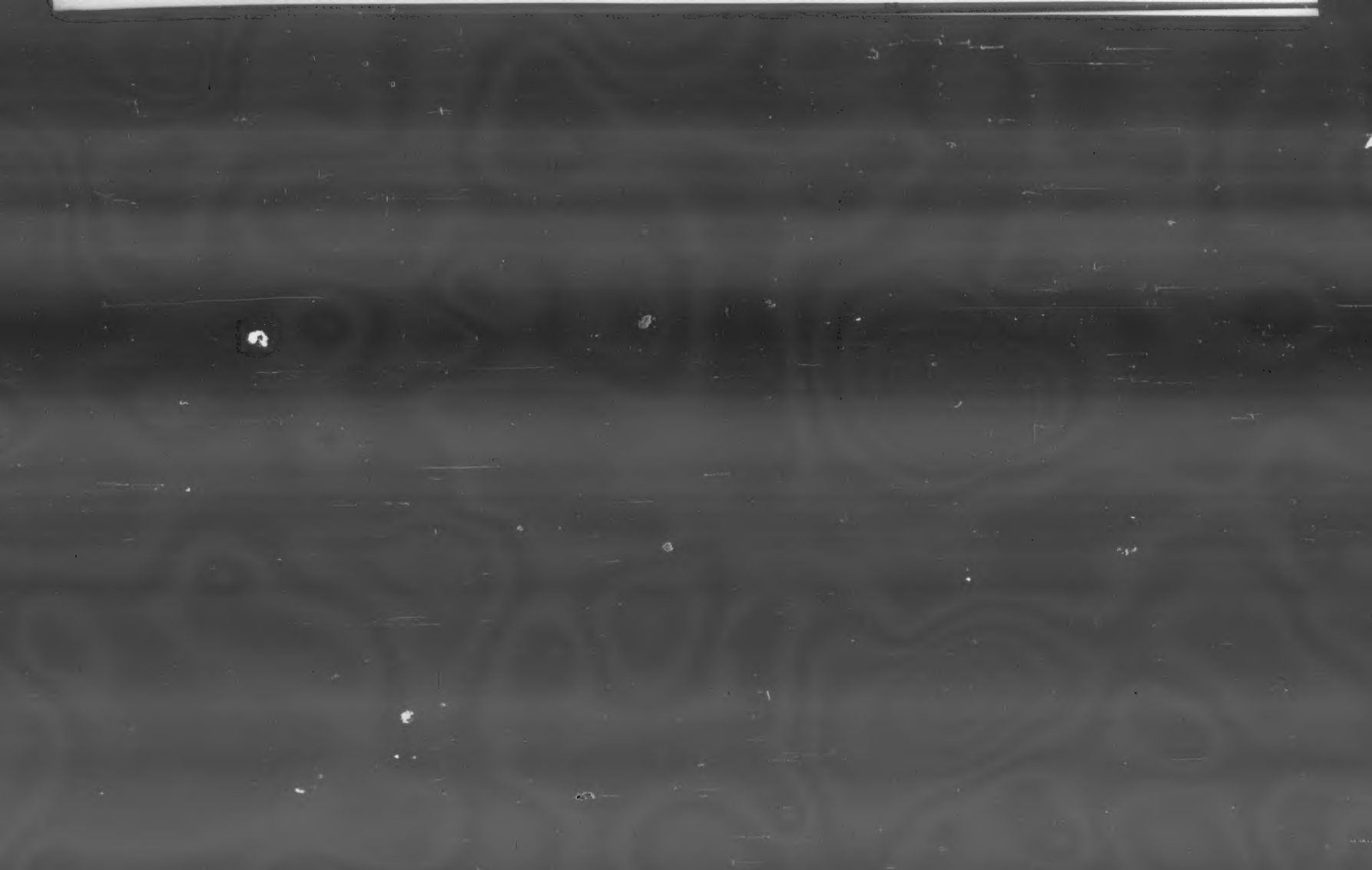
PLATE 25

FIG. 1. Monkey 7. A variety of casts are present in the lumina of the collecting tubules. Many of the tubules show no casts. Hematoxylin and phloxine stain. × 120.

FIG. 2. Monkey 7. Small granules of yellowish brown pigment are present in the cytoplasm of some of the epithelial cells. Many of the lumina are occluded by the swollen cells. Hematoxylin and phloxine stain. × 300.

FIG. 3. Monkey 7. The epithelial cells lining the convoluted portion of the renal tubules are markedly swollen. No significant changes are present in the glomeruli. Mallory connective tissue stain. × 120.





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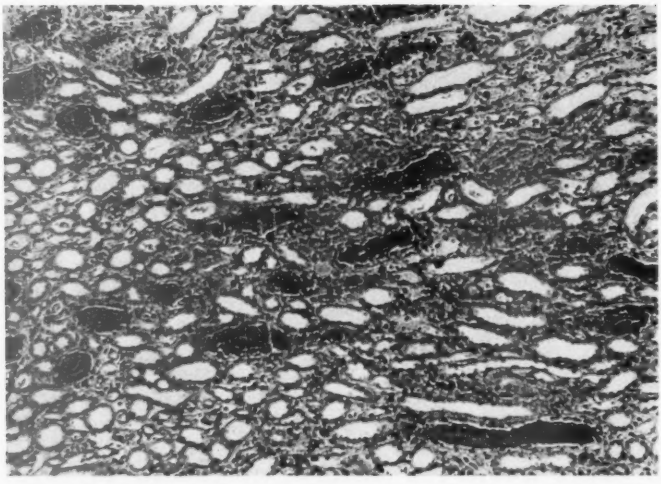

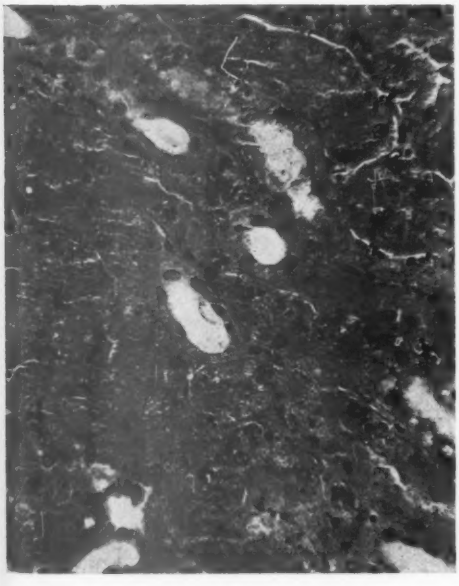

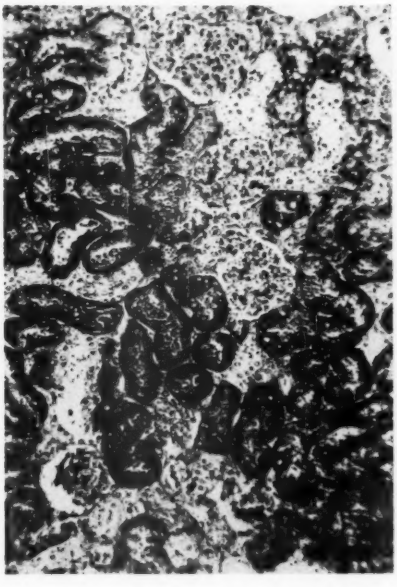

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PLATE 26

FIG. 4. Monkey 29. Casts are present in the lumina of the convoluted portions of the renal tubules. Hematoxylin and phloxine stain. × 120.

FIG. 5. Monkey 29. Some of the casts are formed by pink-staining granules that vary in size, while others are pink-staining and homogeneous in consistency. Hematoxylin and phloxine stain. × 300.

FIG. 6. Monkey 29. Swollen and granular epithelial cells may be noted in the convoluted portion of the renal tubules. Hematoxylin and phloxine stain. × 300.

FIG. 7. Monkey 29. Some of the lumina of the collecting tubules are filled with casts. The epithelium in such areas sometimes has desquamated. Hematoxylin and phloxine stain. × 120.

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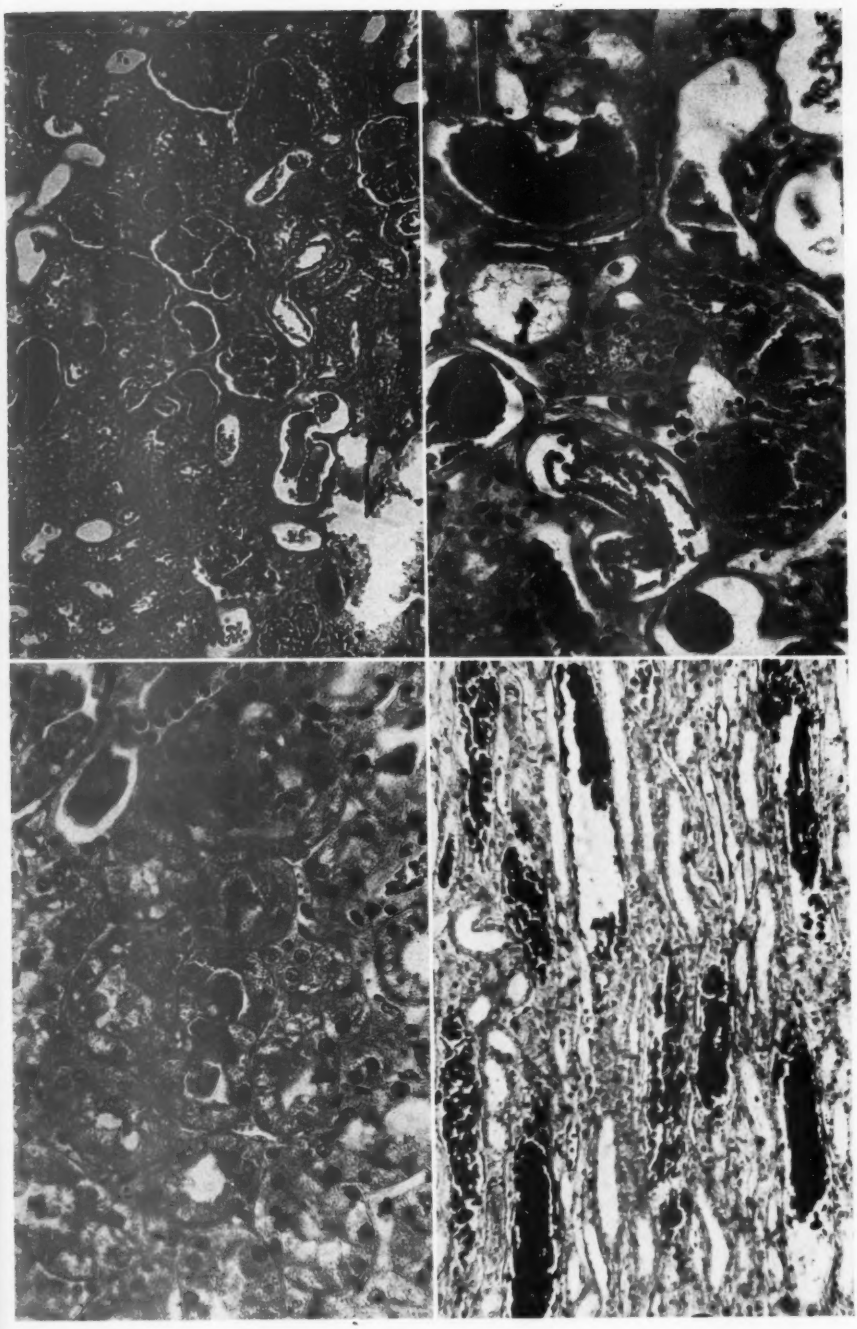

Rigdon Blackwater Fever in Monkeys

ENLARGEMENT OF THE BRONCHIAL ARTERIES, AND THEIR ANASTOMOSES WITH THE PULMONARY ARTERIES IN BRONCHIECTASIS *

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The double arterial blood supply of the lungs has attracted interest and discussion since its discovery. Branches from the aorta to the bronchi were known to Galen, but they were generally forgotten, and even denied by some, such as Columbo, until rediscovered by Dominico de Marchettis, and later by Ruysch.[1,2] There has been controversy concerning the existence of communications between the two circulations; some, like Küttner,[3] maintaining their presence, and others, like Cohnheim and Litten,[4] vigorously denying this. The confusion has been resolved to the satisfaction of most anatomists by the careful work of W. S. Miller [2,5] and of Ghoreyeb and Karsner.[6] The former, in man and dog, could find no precapillary communications between the two systems. When Miller injected the pulmonary artery with a gelatin suspension of Berlin blue, it passed through the capillaries into the pulmonary veins, but not into the bronchial arteries. When the pulmonary venous pressure was increased by clamping the pulmonary vein, there was partial injection of the bronchial artery, through the capillary networks along the respiratory bronchioles. When the injection was carried out through the pulmonary vein, the pulmonary artery was completely, and the bronchial arterial system partially, injected. When the pulmonary artery was clamped, the bronchial arterial system was completely injected from the pulmonary vein, and the pulmonary artery could then be injected from the proximal end with a contrasting material, lead chromate in gelatin. The differences in interpretation of injection experiments previous to those of Miller probably resulted from failure to correlate the results of injections with the finer anatomic detail of cleared specimens observed as three-dimensional objects. Some of the confusion resulted also from the fact that the bronchial artery is the source of the vasa vasorum for the pulmonary vessels. Furthermore, the anatomists, when dealing with human material, often failed to take cognizance of the effects of disease of the lung which may produce profound changes in the circulation. Braus,[7] although claiming the existence

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of anastomoses in normal material, stated that they are much more common in diseased specimens.

A most revealing study of the bronchial artery in disease has been made by Wood and M. Miller of Stanford.[8] They used Hill's mass (bismuth oxychloride in gum acacia).[9] This material was injected into the bronchial artery and roentgenograms were taken of the lungs in their inflated state. Enlargement of the bronchial arteries and numerous large anastomoses with the pulmonary artery were noted especially in subjects with tuberculosis, but also in association with chronic passive congestion, Ayerza's disease, silicosis, emphysema, asthma, and "congenital cystic disease" of the lung. One case of bronchiectasis was mentioned by Wood and Miller. In this specimen the main bronchial artery was described as being slightly dilated and quite tortuous.

In the observations to be reported it was especially in lungs showing bronchiectasis that the most notable expansion of the bronchial arterial circulation was evident. The anastomoses were of such great size and number as to suggest that they possess physiologic importance.

Materials and Methods

Eighteen surgical specimens were prepared as vinylite bronchovascular casts by a method that has been described in detail elsewhere.[10] The bronchial arteries were identified during the course of lobectomy or pneumonectomy for bronchiectasis by their intimacy with the walls of the bronchi, tortuosity, vigorous pulsations, relatively thick walls, and content of bright red blood. They were tagged with longer sutures than the other vessels. The largest vessels were cannulated, washed with water followed by acetone, and were then injected with black plastic. After this procedure the pulmonary arteries and veins were cannulated and the lungs were inflated in a vacuum jar. Red plastic was injected into the pulmonary artery and green plastic into the pulmonary vein through tubes brought out through the cover of the jar. As this material was in the process of hardening, and while the lungs were maintained in their distended state, white plastic containing lead chromate or carbonate was introduced in a similar fashion into the respiratory tree, at first by the negative pressure within the lungs, and then under positive pressure from an injection chamber. After hardening of the plastic, sections for histologic study were cut from significant portions of the specimen. In many instances roentgenograms were made for correlation with the clinical bronchograms. Finally, digestion of the tissue was carried out in concentrated hydrochloric acid. A cast then remained of the respiratory and vascular trees.

Observations

Incidence of Enlargement of the Bronchial Arteries and Pulmonary-Bronchial Arterial Communications

It was obvious in most instances at the time of the surgical operation that the bronchial arteries were greatly enlarged. During the process of injection of the bronchial arteries, moreover, there was usually found an abundant outflow of the black plastic from the cut ends of the pulmonary arteries at the hilum; it was never seen to issue from the veins. Since the plastic as employed for the injection was much too viscid to penetrate the capillaries, this observation at once suggested the existence of sizable anastomoses with the pulmonary arteries. When the casts were examined after injection, striking bronchial arterial enlargement and anastomoses with the pulmonary artery were found in 15 of the 18 specimens. In 11, large branches of the bronchial artery had been identified during the operative dissection or after removal of the specimen, and these were cannulated, washed, and injected directly. In 4 specimens, however, the bronchial arteries were not identified as such, until after injection of the pulmonary arteries from which they had been injected retrogradely. This was to be expected, since the reverse, injection of the pulmonary arteries from the bronchials, was commonly observed, as has been stated. The result was the same as when the arteries were injected separately, except that the objective of demonstrating the two systems in contrasting colors was not achieved. Nevertheless the bronchial vessels were easy to identify by criteria that will be detailed. An example of retrograde injection of the bronchial arteries from the pulmonary side is illustrated in Figure 1. Here the arterial system appears to be at least doubled and often many times compounded for each branch of the respiratory tree, in contrast to the simple arborization of the normal pulmonary arterial tree, where a single branch follows the course of each bronchus or bronchiole.

In 2 of the 3 specimens in which neither direct nor indirect injection of the bronchial arterial system was achieved, the bronchiectasis was of very minor degree. In the third there was severe bronchiectasis of the right middle and lower lobes but anastomoses of the bronchial and pulmonary arterial systems were not demonstrated.

The Bronchial and Pulmonary Arteries Contrasted

In the casts, the bronchial arteries are seen to be more intimately applied to the bronchi than the pulmonary vessels. This is to be expected since the former richly supply the mucous membrane and other elements of the walls of the bronchi themselves, while the pulmonary artery does

not yield such vessels nor any terminal branches until the respiratory bronchiole is reached.* The pulmonary arteries pursue a rectilinear course roughly parallel to the branches of the respiratory tree. The bronchial arterial trunks, however, tend to spiral in relation to the long axis of each bronchus. The pulmonary arteries are truly end arteries and communications exist only among the finer capillary networks; on the contrary, even the larger bronchial vessels, especially in bronchiectasis, are arranged in a dense communicating network. Often the communicating branches are as large as the trunks which they unite. They resemble the arcades of the mesenteric arteries (Fig. 2). It is not rare for a bronchial artery to transgress the boundaries even of a segment. This probably results from the course of some of these arteries within the septa, where vessels from adjacent segments may meet. The plexiform arrangement of the bronchial arteries accounts for the impossibility of cutting off the systemic arterial supply of a bronchus.[11] So numerous are the sources of collateral supply, from branches of the subclavian, internal mammary, pericardiophrenic, esophageal, and numerous intercostal and other derivatives of the aorta, that the effect of ligating the larger identifiable bronchial arteries at their sources is always defeated by an immediate overgrowth of the accessory vessels. The major bronchi are supplied at least by pairs of vessels. Even when a compressive ligature is applied to a major bronchus, the deep mucosal vessels, or perhaps collaterals coursing within the septa or as vasa vasorum, will assure the vitality of the distal tissues.

Location and Nature of the Communications Between the Pulmonary and Bronchial Arterial Systems

An attempt was made to relate the sites of anastomosis of the two systems of vessels to the order of branching of the bronchi. For this purpose, in order to avoid ambiguity, the main segmental bronchus of each segment (in the sense of Brock,[12] or of Jackson and Huber [13] as modified by Boyden [14]) was considered to be of the first order, each of its first two subdivisions of the second order, and so on. No anastomoses were found proximal to the third order bronchi within the segments.† The actual observations are presented in Table I. In most instances the

* An injection so fine as to reach these structures, although easily possible with the vinylite method, was expressly avoided by selecting 12.5 per cent material containing lamp black. With this material no vessels of a diameter less than 50 μ were injected; for the most part the injection was coarser than this.

† This is in contrast to what we have observed in congenital pulmonic stenosis. In this condition the tremendously enlarged bronchial arteries may communicate directly with the pulmonary arteries in the region of the segmental bronchi, or even of a lobar bronchus.[15]

anastomoses were first found along branches of the fourth order in relation to the walls of the large bronchiectatic sacs (Fig. 3). These sacculations, in 15 of our 17 cases of saccular bronchiectasis, begin either in the third or fourth orders of branching. Additional anastomoses, usually multiple and of large size, were often found far beyond the last injected sac (Fig. 1). Many of these probably occur within scar tissue; for in histologic sections it is evident that bronchi and bronchioles which

TABLE I

Results of Bronchovascular Vinylite Injections in Bronchiectasis

Specimen	Mode of injection of bronchial arteries	Location of anastomoses			Maximum size of anastomoses
		Lobe	Segment	Segmental branch where first observed	
					mm.
39865	Direct	R.l.l.	Basal	V	>1.0
40279	Retrograde	L.l.l.	Post. basal	IV	0.75
			Mid-basal	V	
40280	Direct	L.l.l.	All	IV	<1.0
40281	Direct	R.l.l.	Ant. basal	IV	1.0
			Post. basal	V	
40282	Retrograde	L.l.l	Mid-basal	V	<1.0
40716	Direct	L.l.l.	Basal	IV	1.0
41089	Retrograde	L.l.l.	Post. basal	V	1.0
			Ant. basal	IV	1.0
41814	Direct	L.l.l.	Post. basal	V	
			Mid-basal	V	>1.0
41855	Direct	L.l.l.	Post. basal	IV	1.0
			Mid-basal	IV	1.0
42688	Retrograde		Ant. basal	VII	1.0
42689	Direct	L.u.l.	Apical	V	
			Subapical	V	
			Lingula	IV	<1.0
		L.l.l.	Ant. basal	IV	
			Post. basal	IV	
42690	Direct	L.u.l.	All	IV	<1.0
		L.l.l.	Apical	IV	
42691	Direct	L.u.l.	All	IV	
		L.l.l.	All		<1.0
42987	Direct	R.m.l.	Lateral	VII	<1.0
		L.l.l.	All		
43651	Direct	L.u.l.	Lingula	IV	<1.0
		L.l.l	All	IV	

branch from the dilated sacs often have minute lumina embedded within highly vascular granulation tissue, or else their lumina have become completely obliterated. Indeed it may well be that the atelectasis and fibrosis associated with this process produce the force that expands the bronchi,[16] and that the chief locus of the original disease is not so much in the walls of the present bronchiectatic sacs, as distal to them.

Before the actual junction with the pulmonary arteries is reached the bronchial vessels often spiral in a very remarkable fashion (Fig. 4). Spiraling of the pulmonary artery may occur also, but the coils of the

bronchial vessel are tighter, wider, and more numerous. The bronchial arteries normally pursue a spiral course about the bronchi and when fibrosis of the lung occurs, as a result of the mechanisms described, these spirals are compacted and so are more obvious.

In like manner the contraction of scar tissue from the region of the obliterated small bronchioles toward the walls of the large bronchiectatic sacs probably explains why the anastomoses often occur apparently so close to the hilum, when ordinarily the branches of both systems communicate only by the capillaries about the more distal ramifications—the respiratory bronchioles.

Size of the Anastomoses

In most instances the plexuses of bronchial arteries about a single bronchus communicate at many points with the pulmonary arterial system. At each point the bronchial artery is of the same size or slightly smaller than the pulmonary twig which it joins (Fig. 5). It must be remembered that shrinkage of the vinylite occurs after injection, more in the case of the black plastic used for the bronchial vessels, which was of 12.5 per cent concentration, than of the 28 per cent "filled" material, that usually was employed for the pulmonary arteries and veins. The former shrinks an estimated 10 per cent. In 2 specimens the largest communications exceeded 1 mm. in diameter. In 5 they were 1 ± 0.1 mm. in diameter and in 8 they were smaller (Table I). In the vicinity of the sacs the major bronchial arteries often approached the diameter of the pulmonary arterial trunk at the same level, and this was occasionally true even at the hilar end of a segment. In the instance illustrated in Figure 6 the relative diameters of the bronchial and pulmonary arteries were, respectively, 3.1 and 4.2 mm. These data apply only to vessels easily visible grossly. They suggest a much greater blood supply from the bronchial vessels than from the pulmonary arteries, when the greater pressure impelling the blood in the former is considered.

Mechanism of the Enlargement of the Bronchial Arteries

It remains to inquire in what manner the expansion of the bronchial arterial circulation and its anastomoses is associated with the processes that lead to bronchiectasis. Necrosis with the formation of pulmonary-bronchial arterial fistulas is probably not concerned, since otherwise one would expect to find gross communications between the bronchial arteries and the pulmonary veins. But no instance of such communications was found in the abundant material that came under study (Fig. 7).

At least three changes occur during the development and after the

establishment of bronchiectasis which could not exist without an increased supply of oxygenated blood from the aorta: (1) The most significant is the organizing pneumonitis that, according to Mallory [16] and other observers, usually precedes the bronchiectasis (Figs. 8 and 9). At that time the newly budding capillaries that supply oxygen to the leukocytes and other elements of the granulation tissue may be derived from both systems, and these may join. Thus the peripheral capillary bed of the bronchial artery becomes markedly increased and these vessels enlarge. Similar enlargement of systemic vessels which feed large masses of granulation tissue may be observed elsewhere, and large arterial channels may persist even in a well organized scar. Since the pulmonary and bronchial trunks are immediately adjacent, it is not surprising that certain of the larger vascular channels may bring them into free communication and that these channels may persist after organization is complete. That the bronchial vessels may enlarge promptly even in acute pneumonic processes is suggested by the observations of Mathes, Holman, and Reichert [17] on distemper in the dog. (2) The second change is the considerable hypertrophy of bronchial smooth muscle that occurs in the walls of some of the expanded bronchi in certain cases of bronchiectasis (Fig. 10). (3) The third is the increase in lymphoid tissue which may form huge follicles, both in the walls of some of the sacs and in the proximal bronchi. An example is shown in Figure 11. A persistent large bronchial artery in a healed process is shown in Figure 12.

Physiologic Importance of the Expanded Bronchial Circulation and Its Anastomoses

The enlarged bronchial vessels which exist in the walls of the bronchiectatic sacs may be injured by bacterial agents, a frequent occurrence. The bright red blood under systemic pressure may pour into the respiratory tree with serious or even fatal consequences. A similar explanation has been given by Wood and Miller [8] for the large hemorrhages that may occur in some tuberculous cavities.

The anastomoses between the bronchial and pulmonary arteries account in part for the fact that there is usually little or no desaturation of the systemic arterial blood even in severe cases of bronchiectasis. The pressure in the pulmonary arteries that enter the diseased tissue is increased by their communication therein with the branches of the systemic circulation. Thus there is a shunting of pulmonary arterial blood away from the anastomoses into healthy parenchyma capable of more efficiently oxygenating the contained venous blood. In a patient recently observed at the New Haven Hospital, all parts of the left lung were

involved in bronchiectasis; the oxygen saturation of blood obtained from the radial artery was 94.4 per cent. In this patient the vital capacity was 1080 cc., and bronchospirometry showed an uptake of oxygen by the left lung of only 2 cc. per minute, while that of the right lung was 230 cc. per minute. When prepared as a bronchovascular cast, the left lung showed extreme enlargement of the bronchial arteries and numerous anastomoses of these vessels with the pulmonary arteries. Other factors concerned in shunting of blood within the lung are discussed by Hamilton, Woodbury, and Vogt.[18]

It is obvious that in these cases the output of the left ventricle must exceed that of the right by the amount of blood that passes in the circle: Left ventricle→aorta→bronchial artery→pulmonary vein→left auricle →left ventricle. Some conception of the magnitude of the collateral circulation that may exist in the lung under somewhat similar circumstances is gained from experimental observations on dogs after ligation of the pulmonary artery of one lung. In such animals the blood flow of the ligated side can be estimated during bronchospirometry by applying a modification of the Fick principle.[19] A flow of blood through the bronchial vessels in excess of 900 cc. per minute has been observed 18 months after the ligation, although normally the flow through these vessels does not exceed 27 cc. per minute.[20] It seems possible, from the fact that the bronchial vessels in human bronchiectasis are much larger even than those of dogs on the side of the pulmonary arterial ligation, that the collateral blood flow in the bronchiectatic lungs may well be in excess of 1 liter per minute. In dogs one might expect that this would place a burden on the left side of the heart, yet in such animals Schlaepfer [21] has observed hypertrophy of the right ventricle. Further quantitative studies of this subject are necessary. In patients with bronchiectasis the anastomoses open another avenue for the return of blood which reaches the lung via the bronchial arteries. In the normal lung most of the blood that enters the tissue through the bronchial arteries returns to the heart via the pulmonary veins. In many cases of bronchiectasis, however, the bronchial arteries are so large in relation to the pulmonary arteries and the anastomoses with these vessels are so numerous and wide, that it seems reasonable to suppose that there may actually be a reverse flow through some of the peripheral pulmonary arterial branches toward the heart. This hypothesis, which is based merely on anatomic observation, requires confirmation by measurement of the pressures in each of the pulmonary arteries in severe cases of unilateral bronchiectasis, and by the analysis of blood from each of these vessels. If reverse flow exists, the pressure in the proximal pulmonary

artery of the involved side should be higher and the blood richer in oxygen. If backflow does not occur, it is improbable that unilateral bronchiectasis will produce pulmonary arterial hypertension. This statement is based on the evidence brought forward by Cournand [22] that in man, even when the entire cardiac output is forced through a single lung after pneumonectomy, the remaining capillary bed is sufficiently adaptable so that no increase in pulmonary arterial pressure results. In severe bilateral bronchiectasis, however, when no more than a relatively small amount of intact pulmonary substance may remain, the high pressure transmitted from the region of the anastomoses may contribute to pulmonary hypertension and ultimately to the development of cor pulmonale.

Summary

In 15 of 18 specimens of lung removed surgically from patients with bronchiectasis, and prepared as casts by the vinylite corrosion technic, great enlargement of the bronchial arteries and numerous anastomoses of these vessels with the pulmonary arteries were observed. The communications were multiple and usually occurred in the walls of the bronchiectatic sacs which involved branches of the fourth order, or more distal branches, of the segmental bronchi. In half of the specimens the anastomoses equalled or exceeded 1 mm. in diameter.

The enlargement of the bronchial vessels is associated with the development of granulation tissue during the course of the organizing pneumonitis that usually precedes bronchiectasis, and with the metabolic demands of hypertrophied muscle and hyperplastic lymphoid tissue that are often observed. The anastomoses may represent persistent communicating channels, originating in the granulation tissue, that originally received vessels from both the bronchial and pulmonary arterial systems.

The anastomoses are so large and numerous as to suggest that they have physiologic importance: (a) In shunting pulmonary arterial blood away from the diseased tissue into relatively intact parenchyma, where the pulmonary blood pressure is presumably lower; (b) as a factor producing hypertension in the pulmonary circulation.

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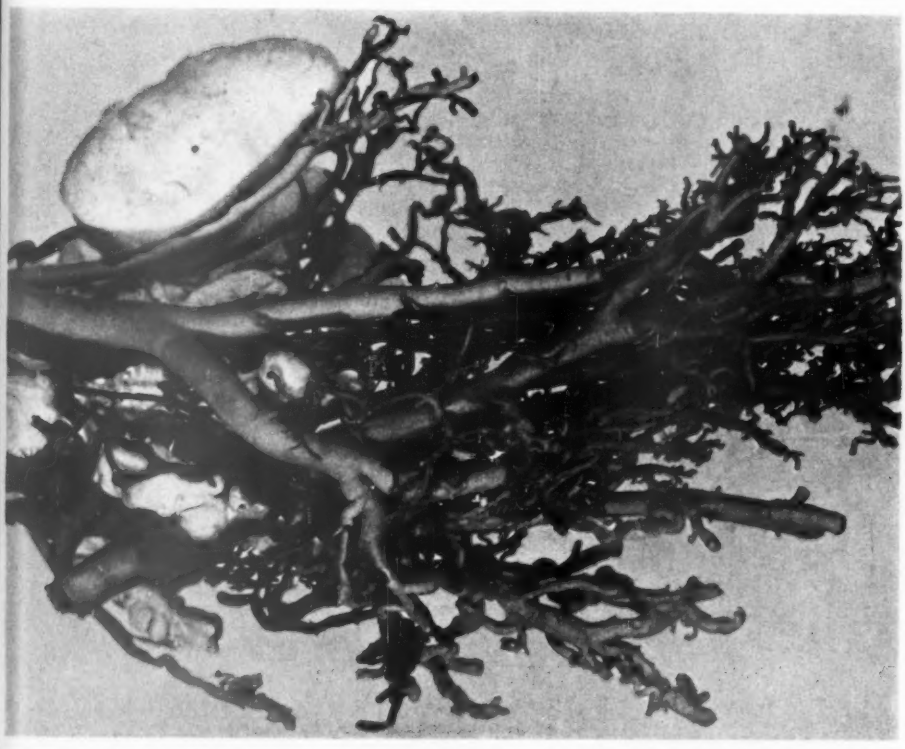
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 27

FIG. 1. Retrograde injection of the bronchial arterial system from the pulmonary arteries. Several branches of the pulmonary artery are shown terminating in the usual arborizing fashion. A bronchial artery (B) is seen below the uppermost pulmonary branch. The bronchial artery has a large anastomosis (AN) with this branch and with at least two other branches of the pulmonary artery. × 2.

FIG. 2. One large pulmonary artery (red) is distributed to each saccular bronchus (yellow) within the segment. In contrast, the bronchial arteries (black) form a dense rete in closer relation to the lumen. Multiple communications exist between the bronchial and pulmonary arteries, best seen among the uppermost branches in the photograph. The veins are injected with green plastic. × 1.75.

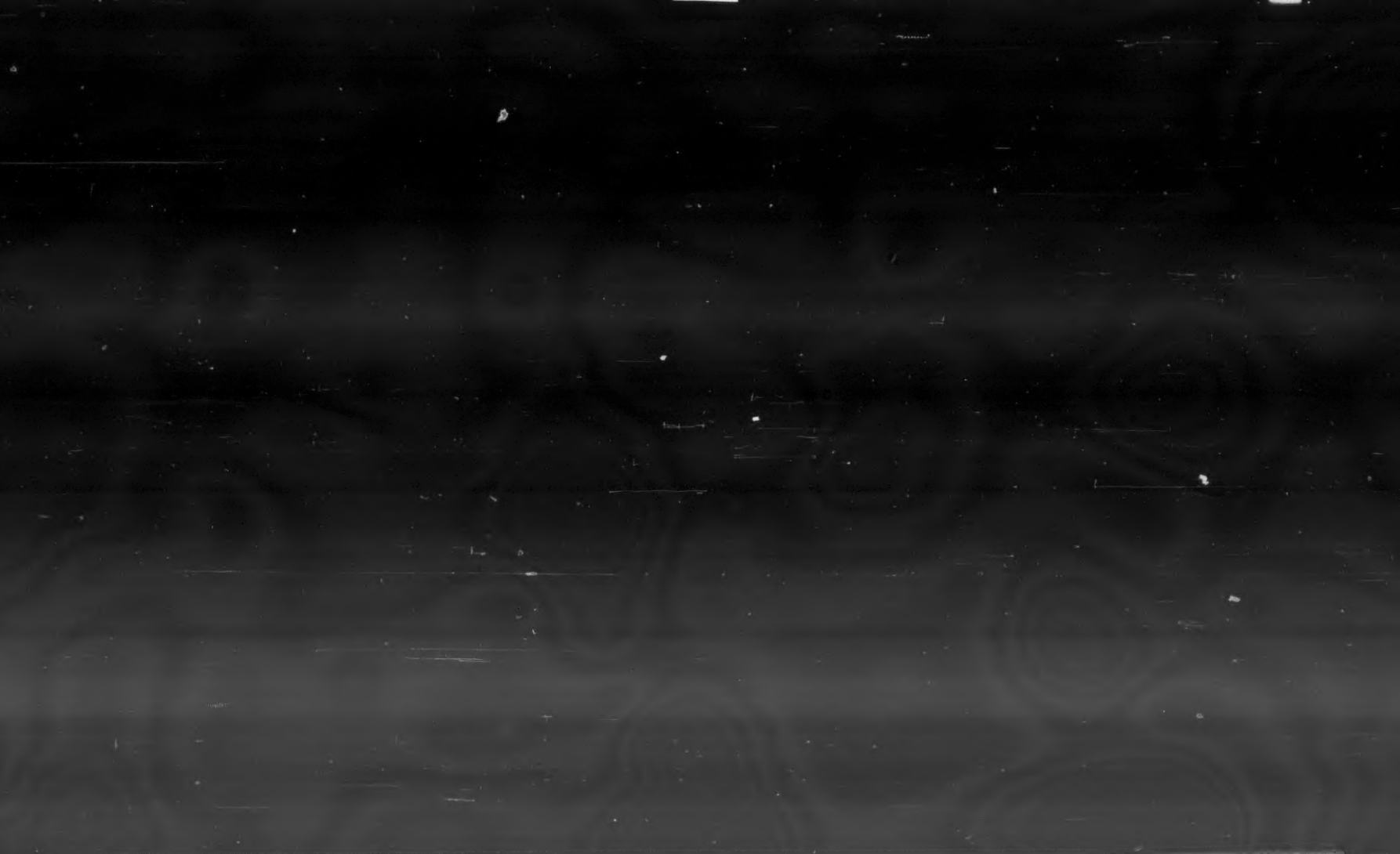


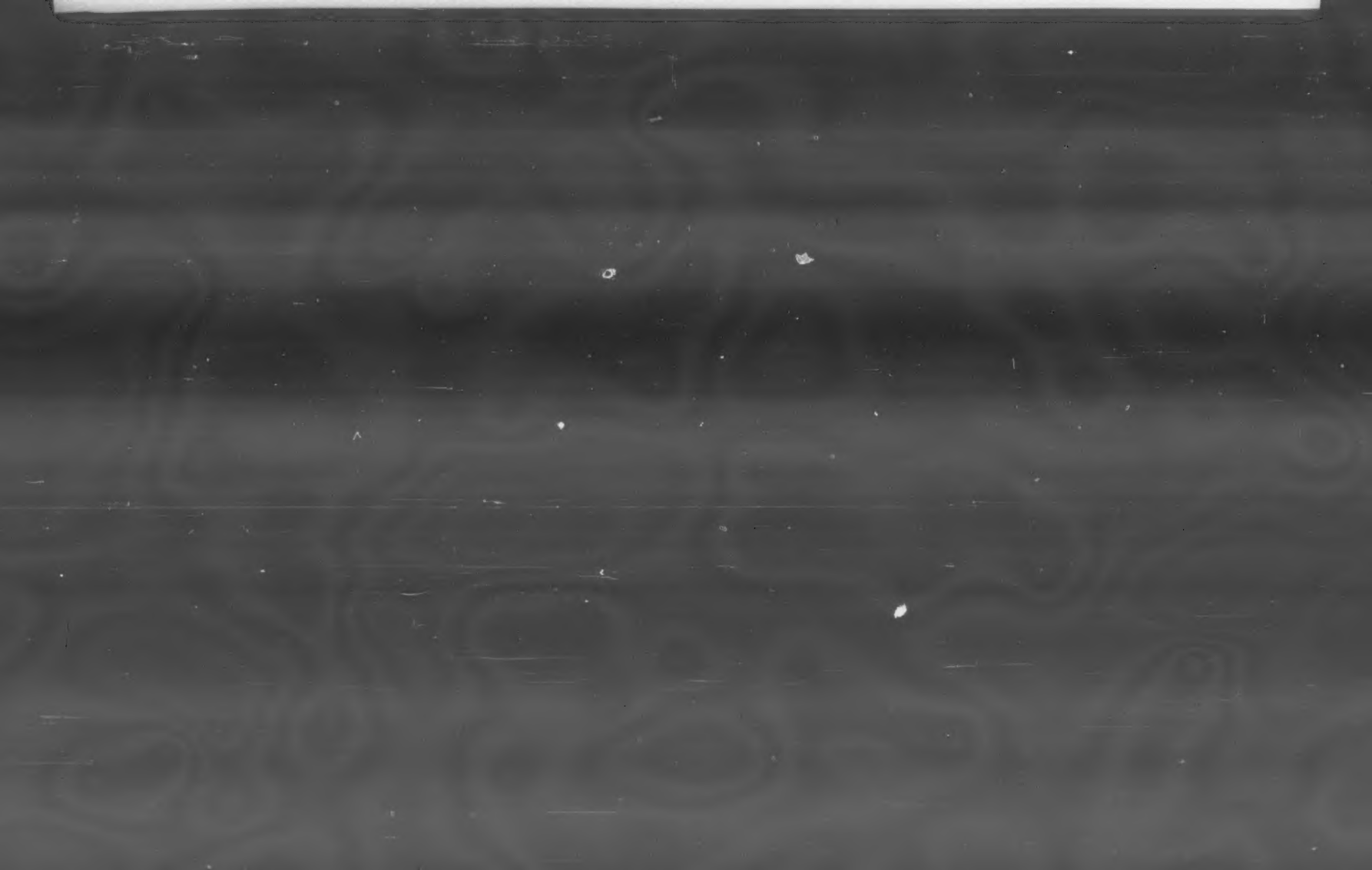
Liebow, Hales, and Lindskog Bronchial Arteries in Bronchiectasis

PLATE 28

FIG. 3. Posterior basal segment of left lower lobe. In relation to expanded, saccular, fourth order branches of the segmental bronchus, a series of branches of the bronchial artery come into communication with branches of the pulmonary artery. Three anastomoses are clearly seen at the left in the photograph. Actually at least five are visible in the original specimen. The plexiform arrangement of the bronchial vessels again may be noted. Colors are as before. × 1.5.

FIG. 4. Above the longest of the injected vessels is a branch of the pulmonary artery which communicates (at AN) with a bronchial artery. As the vessels are traced proximally, the latter is seen to spiral about the former and to reappear as a somewhat attenuated vessel. There has been admixture of plastic across the large anastomosis. × 1.75.





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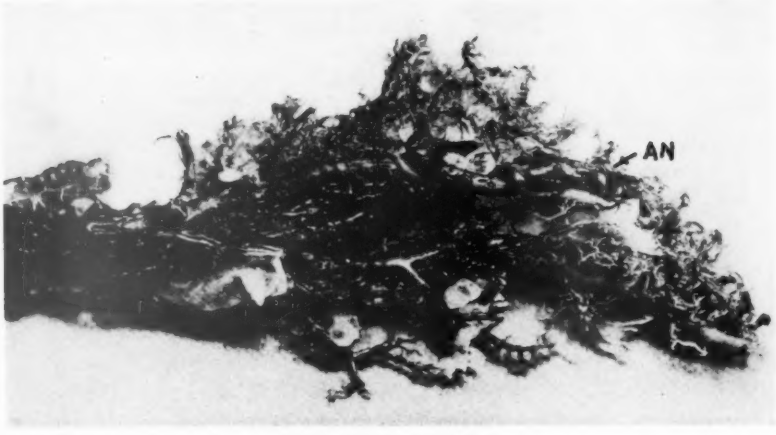
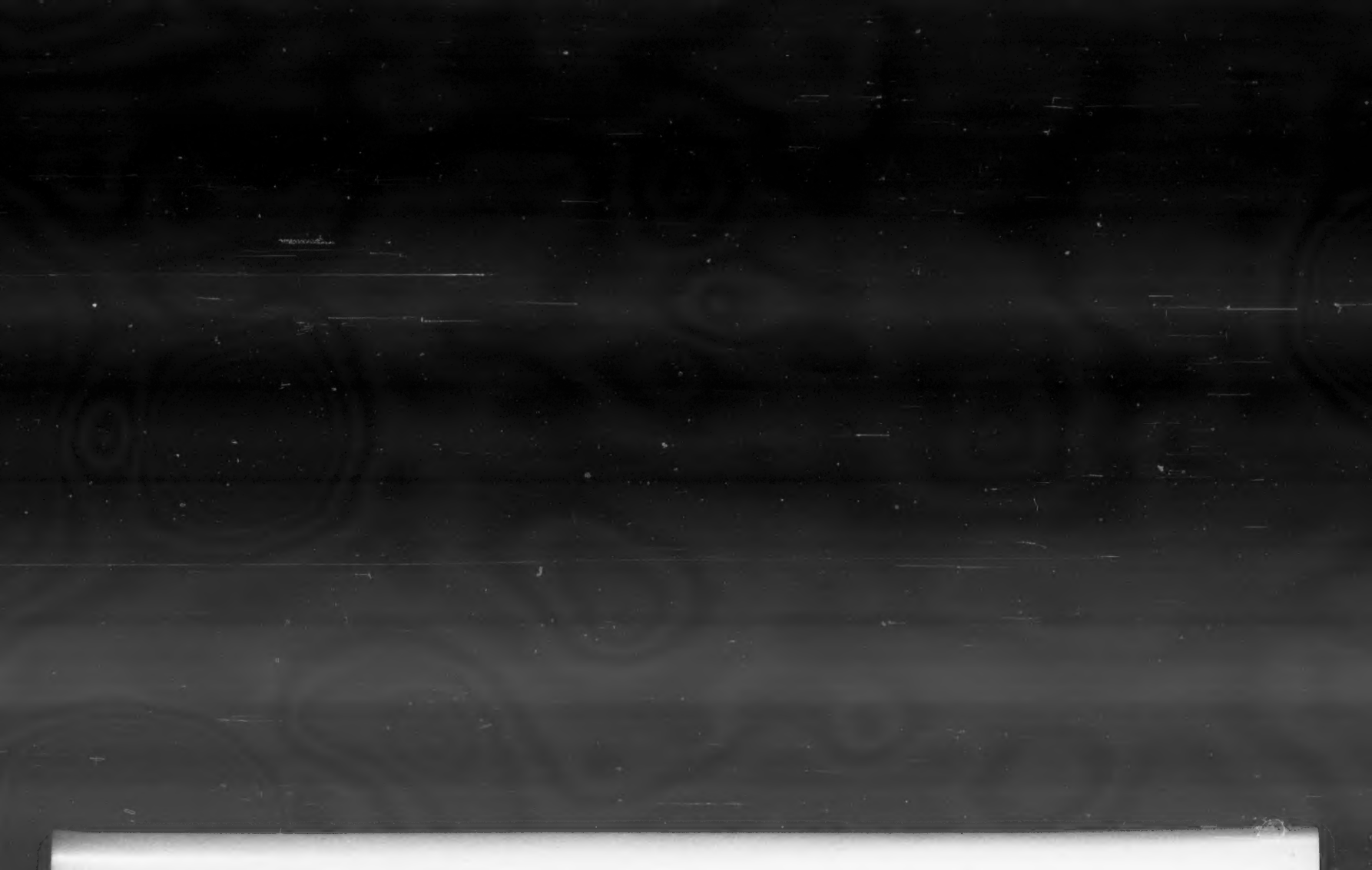


Liebow, Hales, and Lindskog Bronchial Arteries in Bronchiectasis

PLATE 29

FIG. 5. An anastomosis between the bronchial and a pulmonary artery is shown in relation to a bronchiectatic sac. In this instance no visible admixture of the red and black plastics has occurred across the anastomosis. The main bronchial artery is only slightly smaller than the pulmonary arteries of the same subsegment. The anastomosing branches are of approximately equal size. Of note is the more intimate relation of the bronchial artery to the lumen of the sac. × 7.5.

FIG. 6. The large size of the main bronchial artery to a segment is shown. In this instance the bronchial artery seen below the main segmental bronchus at the left has a diameter of 3.1 mm., and the pulmonary artery of the same segment has a diameter of 4.2 mm. In its distal course the bronchial artery divides into a plexus of vessels which, in the original specimen, are injected proximally in black and distally with red plastic from the pulmonary artery by retrograde flow. In the black and white photograph the red plastic appears pale gray. Actual size.



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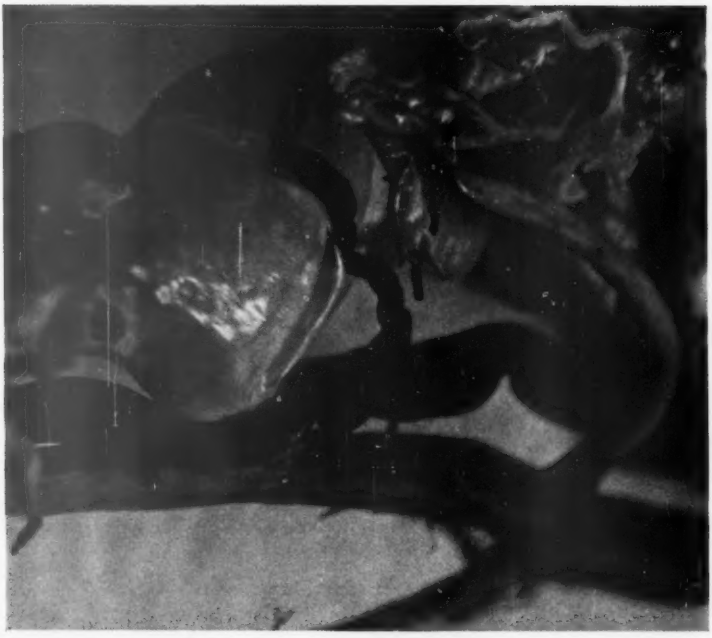

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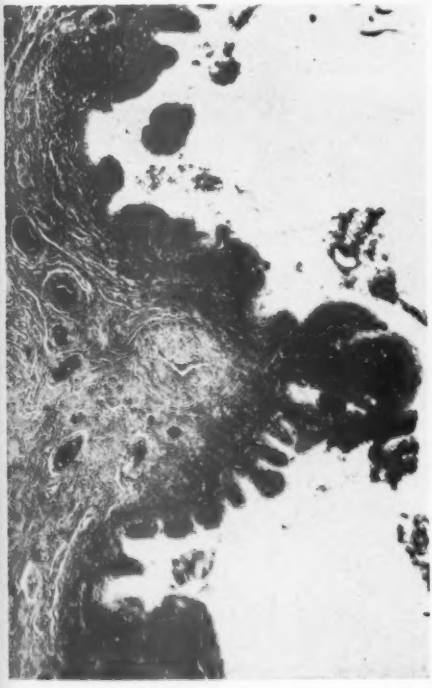
PLATE 30

FIG. 7. The point of confluence of two bronchial arteries with a large pulmonary vessel is shown below the bronchiectatic sac. Some admixture of plastics across the anastomosis has occurred. There is no communication with the pulmonary vein (green). × 4.5.

FIG. 8. A large bronchial artery is seen high in the lamina propria. The trabeculations of the mucous membrane are produced by masses of highly vascular granulation tissue. These are seen under higher magnification in Figure 9. The thick wall and relatively narrow lumen of the bronchial artery may be noted. (41855.) × 32.

FIG. 9. Abundant vascular granulation tissue elevating the pseudostratified ciliated columnar epithelium that lines a bronchiectatic sac. (41855.) × 50.



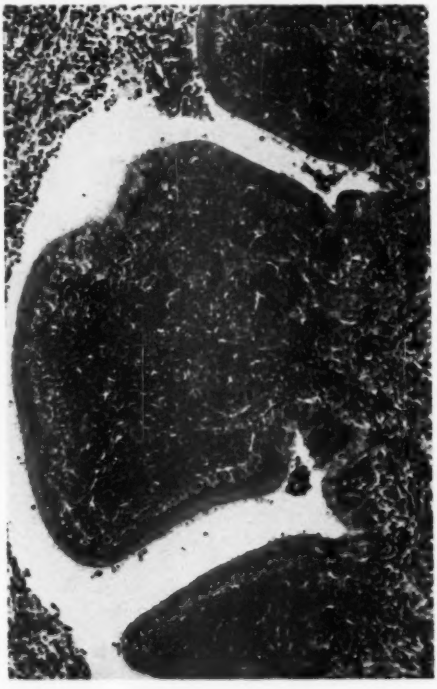
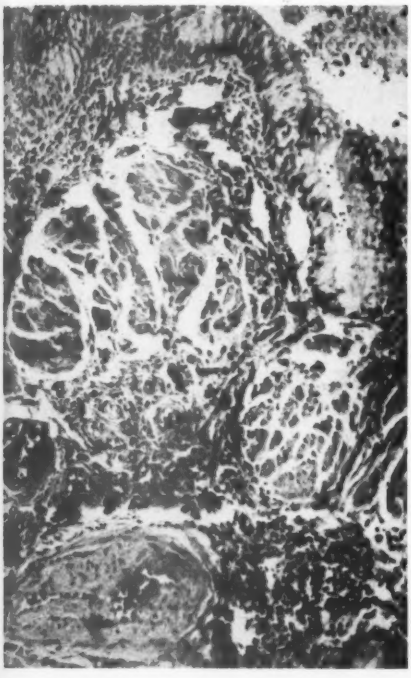


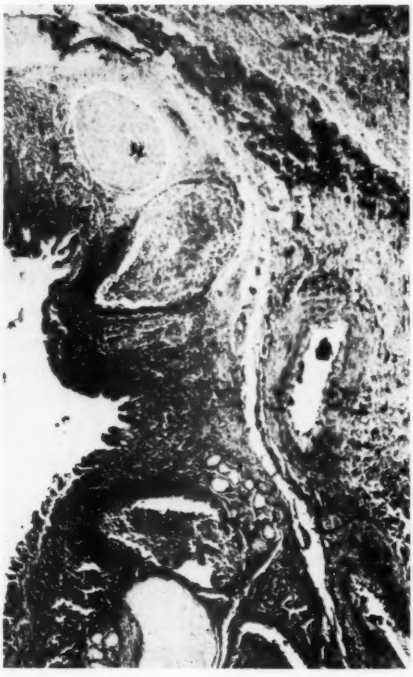
PLATE 31

FIG. 10. Thick bundles of smooth muscle in the wall of a bronchiectatic sac. These receive their blood supply from the enlarged bronchial vessels, one of which is shown near the lower margin of the photograph. (41814.) × 52.

FIG. 11. Wall of a large bronchus in bronchiectasis. Large lymph follicles with prominent germinal centers are seen in the lamina propria. There is also diffuse mucosal infiltration, chiefly of lymphocytes and plasma cells. A large branch of a bronchial artery with a thick muscular wall is seen in the lamina propria. Bound to the external wall of the bronchus is a branch of the pulmonary artery. Pneumonia in process of organization involves the parenchyma surrounding the bronchus. (41910.) × 32.

FIG. 12. Large bronchial artery in the wall of a bronchiectatic sac. An obliquely cut branch traverses the deeper layers of the wall. (41855.) × 50.





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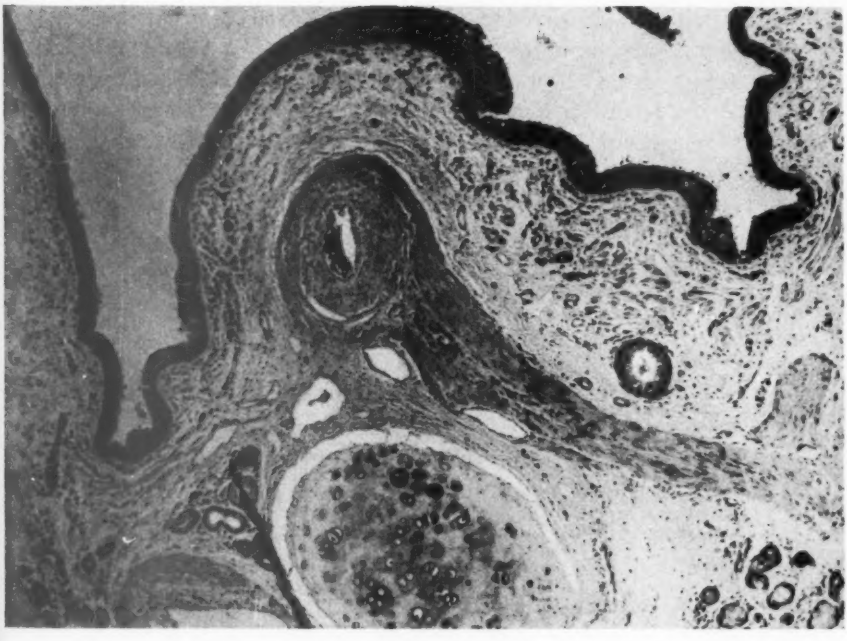

HISTOPATHOLOGIC OBSERVATIONS IN CASES OF HODGKIN'S DISEASE TREATED WITH NITROGEN MUSTARD *

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Several articles have been published [1-9] reporting the clinical response to the use of intravenous nitrogen mustard in health and neoplastic disease. The most dramatic and promising results have been obtained in diseases of the lymphatic tissues, more particularly in Hodgkin's disease. In none of these reports has there been any histopathologic detail given as to the changes in the affected lymph nodes.

The drug used at Walter Reed General Hospital has been the methyl-*bis* (beta-chloroethyl) amine hydrochloride. The following remarks pertain only to the use of the drug in therapeutic dosage, namely, 0.1 mg. per kg. of body weight. Occasionally, larger doses have been used, but in general this amount has been given daily, intravenously, for 4 days.

Although 55 cases (29 of Hodgkin's disease, 6 of reticulum cell sarcoma, 7 of other lymphomata, 5 of myelomata, and 8 of various carcinomas) have been treated at Walter Reed General Hospital in the past 2 years, post-treatment biopsies have not been as numerous as desirable. Nevertheless, such surgical and autopsy tissues as might be expected to indicate changes have been selected and studied repeatedly. In all, 17 cases were chosen as most likely to illustrate any consistent effect of the specific therapy. Ten of these were of Hodgkin's disease, 4 were of reticulum cell sarcoma, and there was one each of chronic lymphatic leukemia, chronic myelogenous leukemia, and transitional cell carcinoma. Sections from all 17 were studied first in unidentified grouping with other sections from cases of untreated Hodgkin's disease and lymphosarcoma, but no selection of the treated cases could be made on the basis of tissue changes.

Pre-treatment and post-treatment sections from known treated cases were then reviewed. Any suspected finding which might be attributable to nitrogen mustard therapy was checked against pre-treatment material from the same case and from untreated cases. By this means the following tentative criteria were studied: (1) The number and concentration of lymphocytes, (2) the formation of secondary follicles, (3) the presence or absence of edema, (4) the mitotic activity in the lymphocyte

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series, (5) the degree of hyperplasia of the reticulo-endothelium, (6) the development of fibrosis, (7) the degree of collagenization, (8) the mitotic activity of the reticulo-endothelium, (9) the relative number of Reed-Sternberg cells and their mitotic activity, (10) the presence of bizarre mitotic figures in any of the actively proliferating cells, (11) the state of the vessels with regard to perithelial or endothelial hyperplasia, necrosis, and obliteration, (12) the presence of cellular degeneration in lymphocytes or reticulum cells, (13) nuclear fragmentation, (14) phagocytic activity with special reference to nuclei and nuclear fragments.

In varying degrees all of these criteria were noted in treated tissues but none was found consistently in the series; in fact, no single criterion can be said to have been seen in any 2 treated cases in which that effect could be attributed to the therapy.

A single case beautifully demonstrated phagocytosis of nuclei and their fragments and is illustrated (Figs. 1 to 3). This node was removed from a case of Hodgkin's disease of sarcomatous type 5 days after a 4-day course of 10 mg. daily. Little gross shrinkage had occurred at that time but considerable shrinkage followed within the next week. However, this feature can be found in no other treated material—the nearest likeness to it is seen in pre-treatment nodes of 2 other cases (Figs. 4 to 6).

Two cases from this series have been reported [8] as showing degenerative changes in the reticulo-endothelial cells. After careful study of both cases we cannot confirm the reported change. Just as poorly preserved and stained reticulo-endothelial cells can be demonstrated in untreated material. In animal experiments heavy dosage produced no such change.[9-11]

Most of the criteria used in this study have been variously reported as having been found following roentgen-ray therapy [12] and in animals rather heavily treated with the various "mustards." [9-11,13-15]

All workers agree that the lymphocytes disappear earliest from the circulating blood after nitrogen mustard therapy and that their loss in the lymph nodes follows promptly. The established short life of the lymphocyte of 12 hours or less,[15,16] with a reserve lower than that for the leukocytes and erythrocytes, would appear to account for this result. There is also a suppression of the activity of that reserve by direct action of the drug upon the depots in the lymph nodes.[9] We have recently seen the shrinkage response of lymph nodes delayed for several days in a case of lymphatic leukemia while the pre-treatment white count of 136,100 dropped to about 5,000. Another observation in this same case, in a node removed after 1 day of therapy, was an apparent decrease in the amount of intercellular fluid.

We have attempted to find the critical period when there might be a wave of lymphocyte destruction, by securing lymph nodes during the treatment, immediately following the 4-day course and at varying later intervals. It has been estimated that a noninjurious quantity of nitrogen mustard remains in the blood stream at the end of 15 minutes.[11] Functional changes, therefore, may be expected promptly but demonstrable morphologic change may not occur for many hours.[14]

The prolonged clinical remissions in some cases are difficult to understand in the absence of any demonstrable microscopic changes after therapeutic doses. In fact, it is difficult to rationalize the use of a drug, which primarily attacks the lymphocytes, in the treatment of a disease generally considered to be one of the reticulo-endothelium. It is fully recognized that the variegated patterns of Hodgkin's disease, which may vary even from node to node in the same patient, make evaluation of fine changes in detail a difficult problem. It is also recognized that the material studied is small in numbers. It would appear that so powerful an agent, yielding such striking clinical results, even though usually transient, should have been controlled by closer histopathologic study. If such studies were made, their omissions from the published reports would imply a series of inconclusive or negative findings similar to ours.

The experimental results in animals, recently released for open publication, all include descriptions of quite definite histopathologic changes, but it must be remembered that the doses were generally large and the response was in other than human tissue. Such results must not be inferred as occurring in the human patients being treated with much less toxic therapeutic doses. It is with this warning idea in mind and in the hope that further observations on human material may be recorded, that the present brief report is submitted.

We wish to acknowledge the assistance of Capt. E. M. Greenspan, M.C., whose clinical studies were made available to us.

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* Search of the current literature fails to find open publication to date. "PB" number refers to the file identification at the Office of Technical Services, U. S. Dept. of Commerce, Washington 25, D.C. Photostatic or microfilm copies may be obtained from that source or The Army Medical Library. A full list of medical reports now released from the "classified" list is given in the Current List of Medical Literature, Vol. 12, No. 7-B, of February 28, 1947.

DESCRIPTION OF PLATE

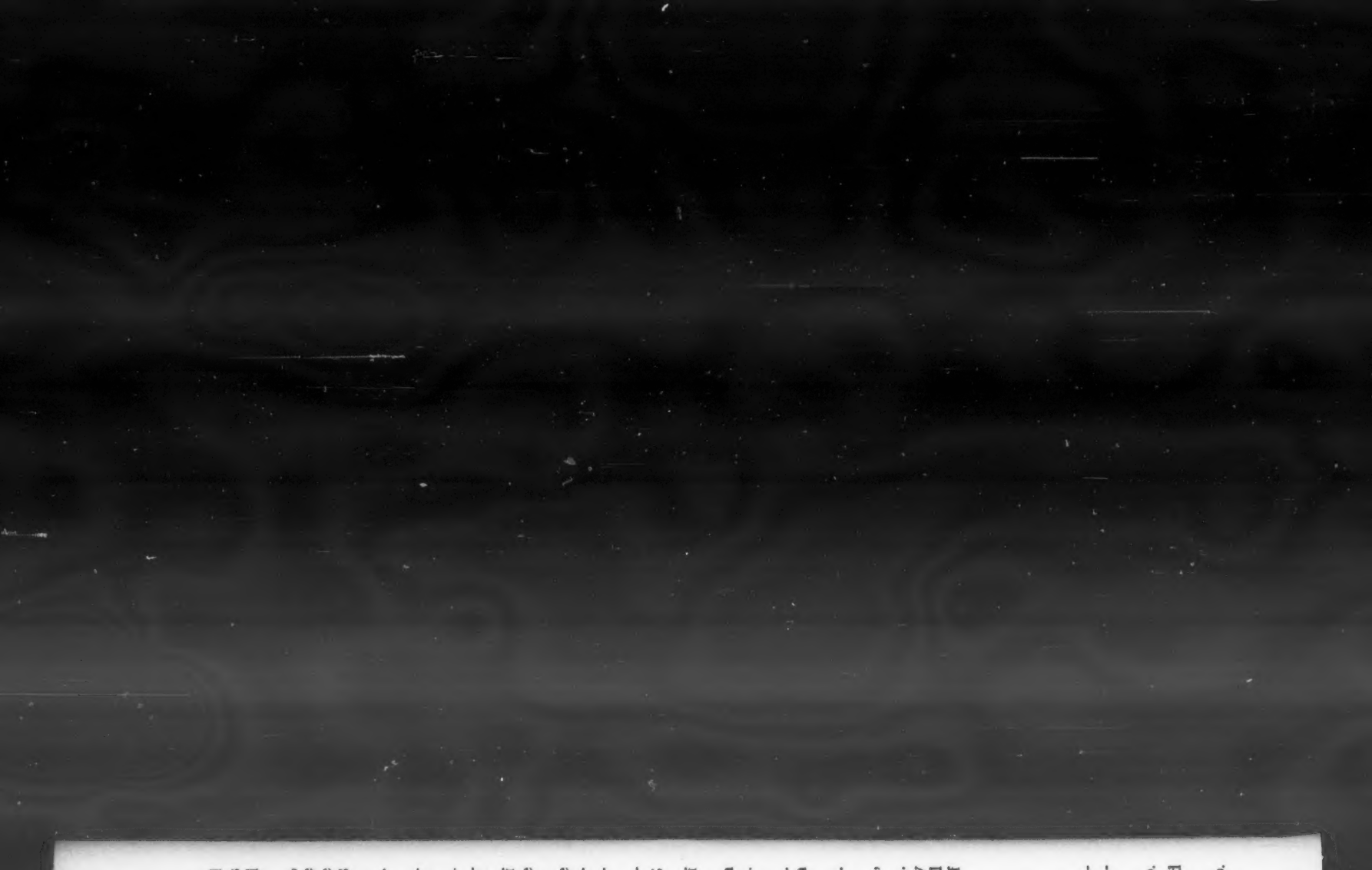
PLATE 32

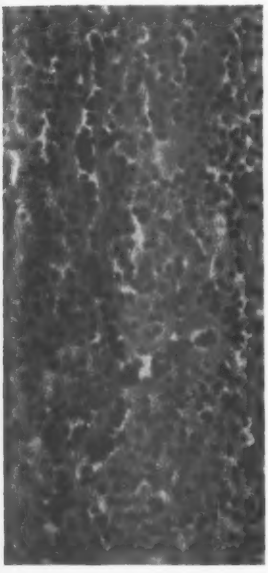
FIGS. 1 and 2. Lymphatic channels in a node with Hodgkin's disease, showing phagocytosis of lymphocytes and their nuclei 5 days after a course of nitrogen mustard. × 140.

FIG. 3. Lymphocytic phagocytosis in the same node at a higher magnification. × 750.

FIG. 4. The same phenomenon as in Figures 1 to 3, but from a node of an untreated case of Hodgkin's disease. × 140.

FIGS. 5 and 6. Lymphocytic phagocytosis in the same node used for Figure 4. × 750.





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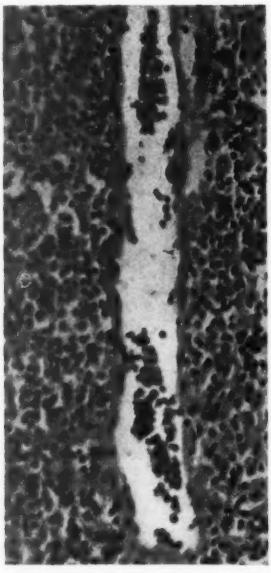

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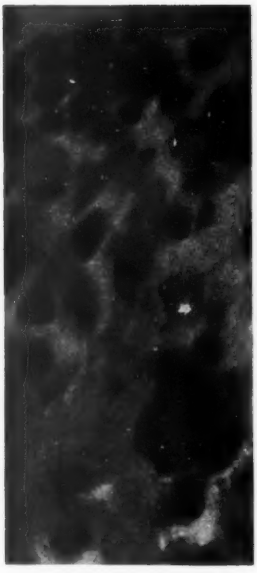

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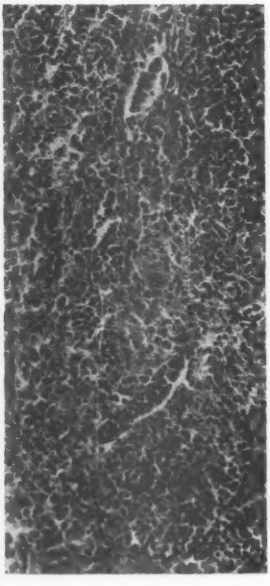

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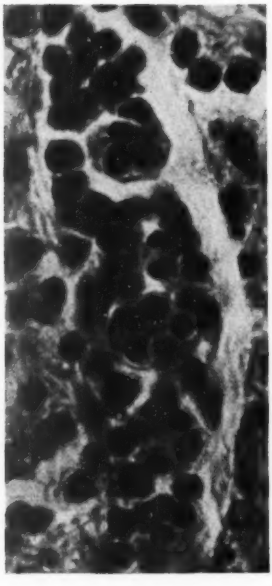

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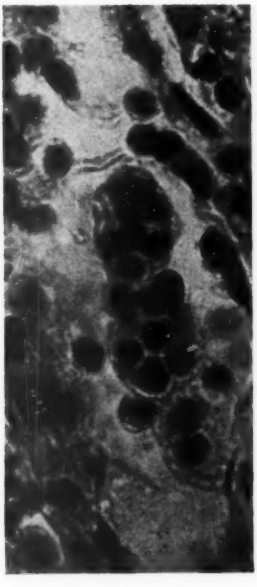

6

CELLS OF THE MEGAKARYOCYTE SERIES IN PERNICIOUS ANEMIA: IN PARTICULAR, THE EFFECT OF SPECIFIC THERAPY *

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Few observations have been published of the morphologic characteristics and numbers of megakaryocytes in the bone marrow of patients with pernicious anemia. Gáspár [1] (1926) found in such patients that the number of megakaryocytes was slightly diminished and that certain degenerative changes occurred in the individual cell. According to this author, the nucleus first lost its fine chromatin network and stained more intensely. It then became somewhat angular and still later stained in a homogenous manner. Next, the nucleus changed into spherical forms of different sizes or into "formlosen" pieces, but remained joined in one mass. Finally, the nucleus seemed to disappear completely. As the nucleus became angular the cytoplasmic granules stained less intensely and then progressively lost their color until they became imperceptible. Fontana [2] (1928) stated that megakaryocytes were extremely rare and even absent in some cases. Tempka and Braun [3] (1932) described megakaryocytes consisting of nuclei for the most part devoid of cytoplasm. Segerdahl [4] (1935) found the number of megakaryocytes to be diminished, and Dameshek and Valentine [5] (1937) also stated that they were decreased in number or completely absent in the bone marrows of patients with pernicious anemia. These authors did not describe any qualitative changes.

Jones [6] (1938) described the megakaryocyte of pernicious anemia as having a coarse, polymorphous nucleus and an intensely basophilic cytoplasm, devoid of azurophilic granules and having streaks of hyaloplasm and irregular areas of spongioplasm. Rohr [7] (1940) found decreased numbers of megakaryocytes in the marrows of patients with pernicious anemia. These cells were described by him as being larger than normal, with marked nuclear segmentation and mature, but still basophilic, cytoplasm which contained few granules. Japa [8] (1943) described normal megakaryocytes as multinuclear cells which develop from unicellular sources by repeated, mitotic nuclear division without cytoplasmic division. The author classified the megakaryocytes on the basis of the number of nuclei present in the cells. He described five groups with 2, 4, 8, 16, and 32 nuclei occurring in frequency in the respective percentages of 2.5, 25.5, 53.0, 18.0, and 1.0. In a case of pernicious anemia he found

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a relative increase in the number of megakaryocytes having 2 and 4 nuclei and a relative decrease in the number of cells having 8, 16, and 32 nuclei. Paseyro [9] (1945) also described increased segmentation, without separation, of the megakaryocyte nuclei in cases of pernicious anemia.

Method, Material, and Definitions

The opportunity of studying the bone marrow of a small group of patients with pernicious anemia before and after clinical and hematologic remissions has recently been available. Differential megakaryocyte counts revealed certain morphologic and numerical changes in the cells of this series, as reported in the present communication.

Five patients were studied, all of whom had clinical and hematologic changes typical of pernicious anemia. Cases 1 and 2 had been on initially ineffectual therapeutic regimens for 20 and 24 days, respectively. Thereafter, 15 U.S.P. units of purified liver extract were administered intramuscularly daily and a characteristic remission was induced in both patients. Case 3 received pteroyldiglutamylglutamic acid, 6.4 mg. daily intramuscularly (containing 4.0 mg. of potential pteroylglutamic acid) for a 14-day period, with a slight reticulocyte response. From the 9th to the 16th day, 15 U.S.P. units of purified liver extract were given daily in addition to the pteroyldiglutamylglutamic acid. This resulted in a satisfactory clinical and hematologic response. Case 4 received 15 U.S.P. units of purified liver extract every second day for a 10-day period and twice weekly thereafter for the next 3 weeks. Case 5 was given 15 U.S.P. units of purified liver extract daily for 7 days, then every second day for 10 days, and twice weekly for 2 weeks. Cases 4 and 5 responded in typical fashion.

Counts of the platelets in the peripheral blood were made with a modification [10] of the direct method described by Rees and Ecker.[11] In my experience, the range of normal variation has been from 150,000 to 400,000 per cmm. Usually two specimens of bone marrow were obtained by aspiration from each patient. The first was taken before treatment was begun; the second, after the reticulocyte response had occurred and the numbers of platelets in circulation had increased. In case 1, the first marrow specimen was obtained after transfusion of the red cells from 1000 cc. of blood-bank blood, but before liver extract was administered. Approximately 0.2 cc. of marrow fluid was aspirated from the sternal marrow cavity with a Turkel needle and small portions of the fluid immediately spread on glass slides. These were permitted to dry in air and later were stained with Wright's stain. The method described by Dameshek and Miller [12] for estimating the number of megakaryo-

cytes in the marrow was employed. This involves counting the number of megakaryocytes present in an area of the preparation and expressing the result in terms of a million nucleated cells. The count is facilitated by accurately determining the number of nucleated cells in 20 oil-immersion fields.

Studies of the marrows obtained before treatment revealed many multinucleated as well as mononuclear cells of the megakaryocyte series. The term *polykaryocyte* has been applied to cells of the megakaryocyte series that contain multiple nuclei in a single cytoplasmic mass. For the identification and classification of these multinucleated cells the following nomenclature and criteria were used.

Young Polykaryocyte. A young polykaryocyte is a multinucleated cell containing 2 or 4 nuclei (Fig. 1). These nuclei have a fine chromatin network and may occasionally contain a nucleolus. The deeply basophilic cytoplasm is homogenous in appearance and may have a few purplish red cytoplasmic granules. Occasionally, portions of the cytoplasm of these cells appeared to be in the process of detachment, possibly in the formation of platelets.

Intermediate Polykaryocyte. An intermediate polykaryocyte may have from 2 to 8 nuclei, but usually has 4 (Fig. 2). The nuclear chromatin is more densely clumped than in the young polykaryocyte, giving it a porous or latticework appearance. Nucleoli are not seen. The cytoplasm is lightly basophilic and may contain from a few to a moderate number of fine purplish red granules. These cells frequently appeared to be forming platelets.

Mature Polykaryocytes. The mature polykaryocyte usually has 6 to 10, occasionally 4 to 18, nuclei (Fig. 3). The chromatin of the nucleus is somewhat more dense and compact than that of the intermediate polykaryocytes. Nucleoli are absent. In some instances 2 or 3 of the nuclei appear to be grouped or fused together while the remainder are distinctly separated in other parts of the cell, as shown in Figure 4. The cytoplasm most often has a neutral or buff color but may at times show a trace of basophilia. Numerous granules similar to those described above are present and platelets appear to be in process of formation by these cells.

The size of the polykaryocytes increases as the cells apparently become more mature. In general, these cells are somewhat larger than the mononuclear megakaryocytes at the same level of maturation.

For the identification and classification of the mononuclear cells of the megakaryocyte series, the following nomenclature and criteria were employed.

TABLE I

Differential Counts by Percentage and Total Numbers of Megakaryocytes

Case no.	Date	Blasts	Mononuclear megakaryocytes									Young	
			Pro-		Lymphoid		Intermediate		Adult		Total		
			Platelet formation		Platelet formation		Platelet formation		Platelet formation			Platelet formation	
			Present	Absent	Present	Absent	Present	Absent	Present	Absent		Present	Absent
1	12-21-46*			3.6		2.4		12.0	2.4	14.4	34.8		
	2-26-47		17.6	1.6	3.2	3.2	8.0	6.4	19.2	16.0	75.2		
2	12-26-46*		7.2			1.2		6.0	4.8	7.2	26.4		
	1-29-47					36.4			36.4	18.2	91.0		
	2-26-47		1.9	1.9		3.8	3.8	24.7	3.8	41.8	81.7		
3	11-29-46*		1.8	1.8	9.0	1.8		12.6			27.0		1.8
	12-24-46		17.6	2.2	4.4	6.6		11.0	13.2	26.4	81.4		2.2
	1-21-47		8.4	12.6	4.2	8.4	4.2	21.0	25.2	12.6	96.6		
4	1-25-47*		41.5		8.3						49.8		
	2-24-47		18.2			1.3	7.8	14.3	16.9	37.7	96.2		
5	4-4-47*		2.5	5.0	2.5		7.5		2.5	12.5	32.5	2.5	
	4-24-47		30.0				5.0	20.0	10.0	25.0	90.0		

* Initial count made prior to the administration of therapy. The remaining counts were made

Megakaryoblast. Megakaryoblasts usually are 20 to 35 μ in diameter. The nucleus usually is round or oval. It has a fine chromatin structure and frequently contains several nucleoli. The basophilic cytoplasm does not contain granules and the cell shows no evidence of platelet formation.

Promegakaryocyte. The promegakaryocyte usually is somewhat larger than the megakaryoblast, although it may be of approximately the same size. The nucleus is oval or partially lobulated. Its chromatin is more clumped than in the megakaryoblasts and nucleoli are not seen. Only a small amount of cytoplasm is present relative to the size of the nucleus. The cytoplasm often contains a few azurophilic granules and in some instances apparent platelet formation may be found at the periphery of the cell.

Lymphoid Megakaryocytes. The size of the lymphoid megakaryocyte usually is somewhat greater than that of the promegakaryocyte. The nucleus is lobulated and relatively small compared to the amount of cytoplasm present. Its chromatin is dense and nucleoli are absent. The cytoplasm is basophilic and usually does not contain granules. Only rarely do these cells seem to be producing platelets.

and Polykaryocytes in the Bone Marrow of 5 Cases of Pernicious Anemia

Polykaryocytes					Degenerated forms					
Intermediate		Mature								
Platelet formation		Platelet formation								
Present	Absent	Present	Absent	Total	Vacuolated	Smudge	Mitotic figures	Total number of mononuclear megakaryocytes and polykaryocytes per million nucleated cells	Total percentage with platelets	Range of platelet counts in peripheral blood (in thousands)
	21.6		36.0	57.6	1.2	8.4		178	2.4	26–68
	1.6	1.6	8.0	11.2		12.8		259	49.6	213–280
4.8	28.8	3.6	25.2	62.4		7.2	1.2	442	20.4	136–190
						9.1		91	36.4	219–281
	5.7		1.9	7.6	1.9	9.5		157	9.5	260–280
	23.4	5.4	37.8	68.4	5.4			670	16.2	54–71
	4.4		2.2	8.8	4.4	6.6		361	37.4	140–248
	4.2			4.2				287	42.0	192–293
8.3		24.9	16.6	49.8				128	83.4	76–98
						5.2		287	42.9	170–230
5.0	30.0	5.0	15.0	57.5	2.5	7.5		147	25.0	66–94
						10.0		100	45.0	200–300

after the institution of anti-pernicious anemia therapy.

Intermediate Megakaryocyte. The intermediate megakaryocyte is considerably larger than the promegakaryocyte. The nucleus contains dense chromatin and shows varying degrees of lobulation. Many granules are present in the lightly basophilic cytoplasm. Intermediate megakaryocytes often appear to form platelets.

Adult Megakaryocyte. The adult megakaryocyte is a large cell, usually well over 40 μ in diameter. Its multilobed nucleus has dense, compact chromatin. The abundant, neutrophilic or slightly basophilic cytoplasm contains numerous azurophilic granules. These granules form into groups surrounded by nongranular cytoplasm at the periphery of the cell. Pseudopodial processes containing these platelet-like bodies are often seen.

Listed under the heading of *smudge* (Table I) are naked megakaryocyte nuclei or remnants of nuclei and cytoplasm that had been damaged during the preparation of the smear. Fragments of cytoplasm occurring without nuclei were not included in these counts.

It is to be emphasized that in the examination of bone marrow one sees numerous transitional forms of all series. Frequently, therefore, an

individual polykaryocyte or mononuclear megakaryocyte may not rigidly conform to the description of the cell type at a particular level of maturation. Such a classification as that described here and elsewhere is of value, however, in that it permits a grouping of cells of approximately the same stage of development for comparison of counts obtained serially from a given patient and those obtained from different sources.

Results

The results of the counts of the cells of the megakaryocyte series are given in Table I. The most significant findings are the changes in the ratios of polykaryocytes and mononuclear megakaryocytes as remissions were induced by liver extract therapy. Before remission, the percentages of polykaryocytes were invariably high, ranging from 49.8 to 68.4, but this increase bore no relation to the initial hemoglobin levels, which were between 4.1 and 10.1 gm. per 100 cc. of blood. In remission only 0.0 to 11.2 per cent of polykaryocytes were present. Conversely, prior to treatment, the percentages of mononuclear megakaryocytes were low, with extremes of 26.4 and 49.8, and in remission were high, varying from 75.2 to 96.6. These changes applied to the absolute as well as to the relative numbers of polykaryocytes and mononuclear megakaryocytes.

In 4 of the 5 patients, the increased number of polykaryocytes found in the initial examination of the bone marrow was associated with low platelet counts in the peripheral blood. In the fifth patient, the platelet counts were in the lower range of normal variation. In each instance as the patient responded to therapy and the platelet counts in the peripheral blood increased, the number of polykaryocytes in the marrow decreased. The total numbers of mononuclear megakaryocytes and polykaryocytes obtained by the initial marrow aspiration varied from 128 to 670 cells per million nucleated marrow cells. Three of these five initial counts were within the normal range of 99 to 270 megakaryocytes per million cells established by Dameshek and Miller,[12] while the remaining two counts exceeded this range. The megakaryocyte counts that were within the range of normal were obtained when there was peripheral thrombocytopenia, and one of the elevated megakaryocyte counts was obtained when there was no thrombocytopenia. In 3 of the 5 cases the percentage of cells of the megakaryocyte series exhibiting apparent platelet formation was less in the initial specimen obtained before treatment than subsequently when remission had occurred. This correlated well with the change in the platelet counts in the peripheral blood. However, in case 2, when the patient was in clinical and hematologic remis-

sion, only 9.5 per cent of the total number of the cells of the megakaryocyte series appeared to be producing platelets. In case 4, when there was peripheral thrombocytopenia, 83.4 per cent of the megakaryocytes apparently were forming platelets; subsequently when remission had occurred and the platelet counts were normal, only 42.9 per cent of the megakaryocytes appeared to be forming platelets. Thus, in these studies there seemed to be no constant relationship between the platelet counts in the peripheral blood and the total number and percentage of cells of the megakaryocyte series apparently producing platelets.

Discussion

Di Guglielmo [13] originally expressed the view that the polykaryocyte is an intermediate cell type that develops during the formation of the mature megakaryocyte from fusion of primitive mononuclear cells. This interpretation of the origin of the adult cells of this series is a point disputed by hematologists. Although polykaryocytes are observed occasionally in specimens of bone marrow obtained from normal children and adults, and in the marrows of patients having a variety of diseases of the hematopoietic tissues, they are not of the type of megakaryocyte usually seen.

There is little possibility of confusing the polykaryocytes described above with osteoclasts and multinucleated immature cells of the erythrocyte series. Not only do they have different morphologic characteristics of the nucleus and cytoplasm,[14] but also the cytoplasm of the polykaryocyte contains granules and at times appears to produce platelets, while cells of the other two types do not. Nor can polykaryocytes be considered to be degenerating megakaryocytes since they appear actively to form platelets and do not exhibit features of degeneration, such as vacuolization or hyalinization of the cytoplasm or pyknosis of the nucleus.

In the marrow of the cases of pernicious anemia studied, young, intermediate, and adult forms of polykaryocytes and mononuclear megakaryocytes apparently coexisted. Before treatment the polykaryocytes were present in relatively increased numbers, while the converse situation existed in remission. The data presented above indicate that before treatment the increase in the number of polykaryocytes is independent of the hemoglobin concentration in the peripheral blood.

Furthermore, the opportunity was presented by Dr. Charles S. Davidson to review bone marrow preparations made before and after transfusions of whole blood and of red cell concentrates as well as after specific liver extract therapy in patients with pernicious anemia. David-

son, Murphy, Watson, and Castle [15] found that, entirely without specific therapy, transfusions sufficient to elevate the erythrocytes and hemoglobin to normal values caused the disappearance of the megaloblasts from the bone marrow in pernicious anemia, presumably as a result of the elevation of the oxygen-carrying power of the blood. However, the numbers of leukocytes and platelets in the peripheral blood were not increased and examination of the bone marrow smears revealed no significant reduction of the numerous polykaryocytes present before transfusion. Indeed, these cells became decreased or were completely absent only after adequate specific therapy, in the form of liver extract, had been administered. Thus, in contrast to the situation with respect to the megaloblasts, it seems that in pernicious anemia the mononuclear megakaryocyte:polykaryocyte ratio is not readily modified by increase in the oxygen-carrying capacity of the peripheral blood. Instead, it depends on the presence of the anti-pernicious anemia factor(s), the administration of which also diminishes the megaloblasts in the marrow in pernicious anemia prior to significant increase in the oxygen-carrying capacity of the blood.[16]

With the technic of study employed, there was no evidence of decreased numbers of cells of the megakaryocyte series in the marrows of patients before treatment. Indeed, in 2 of the 5 cases the numbers of these cells exceeded the range of normal established by Dameshek and Miller.[12] In the remaining 3 cases the megakaryocyte counts were normal.

Conclusions

Repeated bone marrow studies of 5 cases of pernicious anemia have been made with emphasis on the morphologic appearances and numbers of mononuclear megakaryocytes and polykaryocytes.

Before remission was induced by specific therapy there was an increased number of polykaryocytes and a decreased number of mononuclear megakaryocytes in the bone marrow, while following remissions induced with liver extract, this ratio of cells was reversed.

Evidence is presented to indicate that, in contrast to the situation with respect to the young erythrocyte-forming cells (megaloblasts), a shift in the type of bone marrow megakaryocyte does not occur with an artificial increase in the oxygen-carrying capacity of the peripheral blood as a result of transfusions given without the administration of specific therapy.

A decrease in the percentage of bone marrow polykaryocytes, however, does follow the administration of adequate amounts of anti-pernicious anemia therapy as does also the disappearance of megaloblasts

prior to a significant increase in the oxygen-carrying capacity of the peripheral blood.

The total number of cells of the megakaryocyte series was either normal or increased in the marrows of patients in relapse.

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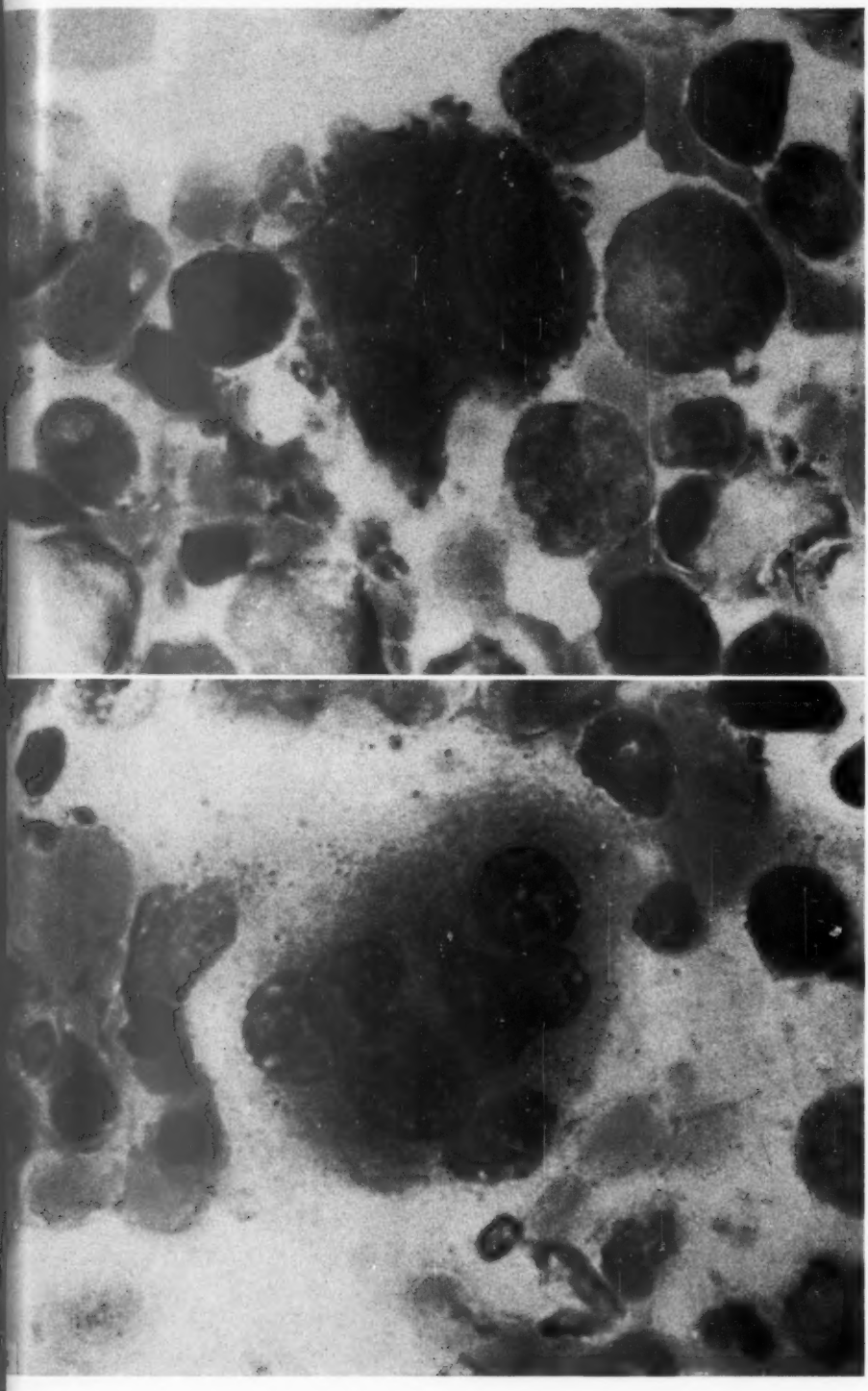
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 33

FIG. 1. "Young" polykaryocyte from case 5. This cell has 4 nuclei containing fine chromatin, and a basophilic cytoplasm in which there are a few scattered granules. A few platelets apparently are being formed at one portion of the periphery. × 1150.

FIG. 2. "Intermediate" polykaryocyte from case 1. The chromatin in these 7 nuclei shows increased clumping. The faintly basophilic cytoplasm contains many small azurophilic granules. There is no evident platelet formation. × 1150.

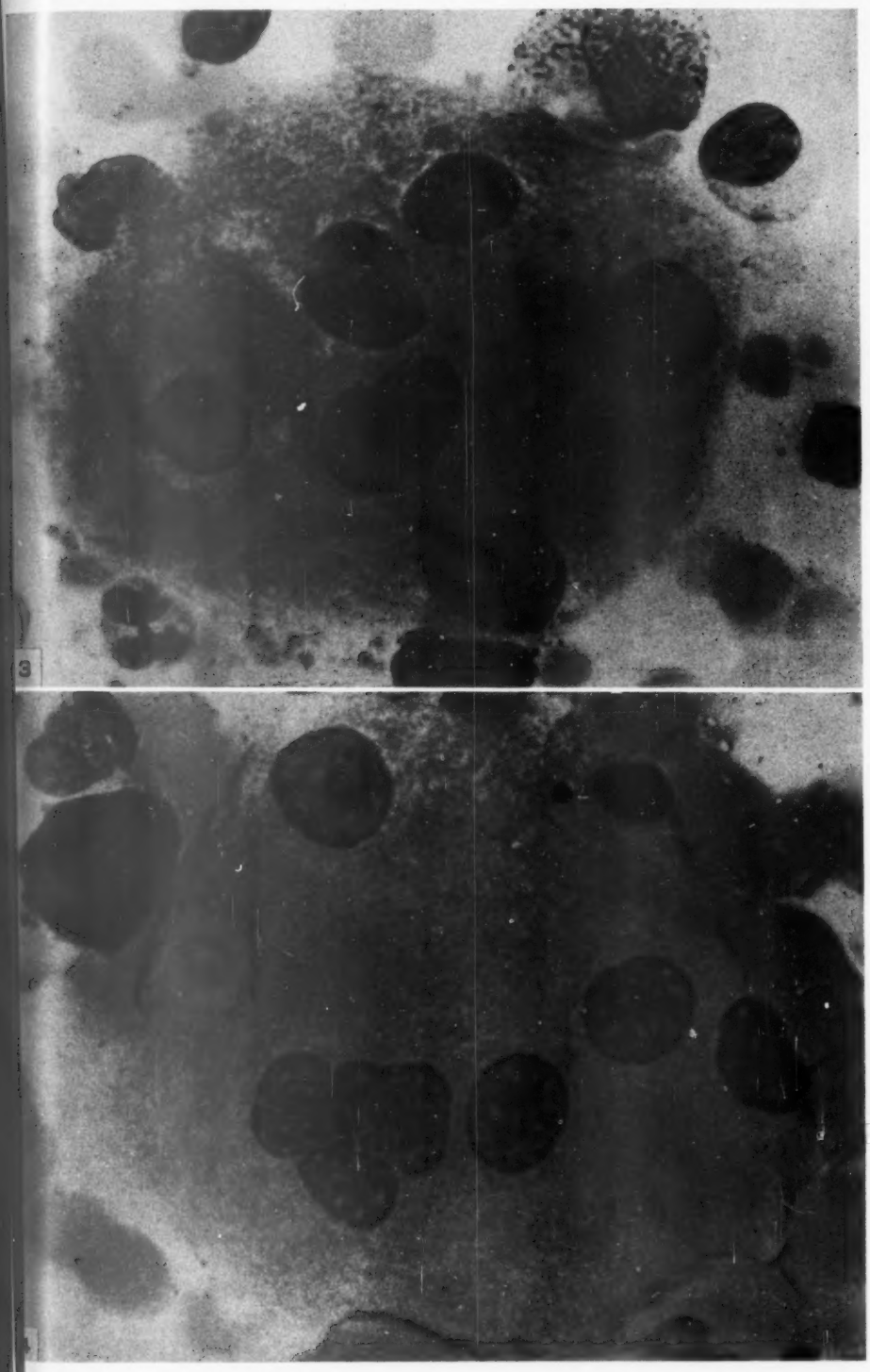


Epstein Megakaryocyte Series in Pernicious Anemia

PLATE 34

FIG. 3. A "mature" polykaryocyte with 7 nuclei from case 2. The nuclear chromatin is slightly more dense and compact than that shown in Figure 2. The cytoplasm is free of basophilia and contains many granules. There is apparently some platelet formation. × 1150.

FIG. 4. A "mature" polykaryocyte from case 3, showing grouping of 4 of the 8 nuclei. × 1150.



Epstein Megakaryocyte Series in Pernicious Anemia

THE EFFECT OF NORMAL SALINE SOLUTION, RINGER'S LACTATE SOLUTION, AND DISTILLED WATER ON THE LUNGS OF DOGS AND RABBITS *

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The original purpose of this study was to determine the effect of amniotic fluid, meconium, and vernix caseosa on pulmonary tissue. Meconium and vernix were suspended in normal saline solution because the latter had been found by Winternitz [1] to produce practically no polymorphonuclear exudate and only moderate capillary congestion and hemorrhage when introduced into the lungs of dogs in large quantities. In all dogs in which either meconium or vernix mixed with normal saline solution or amniotic fluid by itself was introduced into the lungs, we found a marked polymorphonuclear exudate in the alveoli and bronchi between 6 and 30 hours later. To exclude all possibility that this reaction was produced by the normal saline solution, we investigated the effect of this fluid on the lungs of dogs. We found an unanticipated polymorphonuclear exudate when saline solution alone was used, which was just as marked in its initial phase as when meconium and vernix were mixed with saline solution. These results led us to explore also the effect of distilled water and Ringer's lactate solution on the lungs of dogs and of saline solution on rabbits. The present report is concerned with the results of the latter experiments. The effects of amniotic fluid, meconium, and vernix on the lungs of dogs and rabbits will be reported separately.[2]

METHODS

The normal saline solution used in these experiments was obtained from two sources. Ordinary tap water, which in our laboratory is alkaline, was distilled once in a metal still, using stainless steel and block tin as condensers. Following distillation, the water was tested for chlorides with silver nitrate and for available alkali with a methyl red, methylene blue indicator. Solutions containing chlorides or available alkali were rejected. Following the addition of 9 gm. of sodium chloride per l. of water, the solution was autoclaved without preservative being added. Fresh normal saline solution was made up every day. The above solution was used on all dogs numbered from 106 to 145 (Table I) and on all rabbits in Table II. The normal saline solution and distilled water used on dogs 145 through 152 were made by Abbott Laboratories and were pyrogen free and free of preservative. The Ringer's lactate solu-

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tion was made by diluting a 10 cc. ampule of lactate-Ringer's solution, made by Eli Lilly Co., twenty-five times with sterile distilled water (Abbott Laboratories).

Young, healthy, adult dogs and rabbits were used. The animals were given sulfadiazine and penicillin 1 hour or less before the introduction of the fluid into their lungs. Sulfadiazine was given subcutaneously in a 1 or 2 per cent solution in doses averaging ½ gr. per lb. of body weight. The dogs were given 100,000 units of penicillin in emulgen intramuscularly and the rabbits received half of this amount. If the experiment lasted 18 hours or longer, sulfadiazine and penicillin were given twice a day in the same amounts as originally administered.

Various anesthetics were used. Either nembutal intraperitoneally plus morphine sulfate intramuscularly or sodium pentothal intravenously was employed to induce surgical anesthesia. Both successfully abolished the cough reflexes of all animals. A few experiments were done using only a local anesthetic. After the skin of the neck had been infiltrated with 2 per cent novocaine, the trachea was exposed through a small incision and the solutions were introduced into the lung through a needle inserted between the tracheal rings.

All solutions were introduced either through a bronchoscope passed through the mouth into the trachea with its tip directed into the right or left main bronchus, or through a needle inserted into the trachea as described above. All solutions were sterile and in all experiments a sterile technic was employed, so that contamination of the lung was avoided wherever possible. The solutions were introduced into the lungs while the animals were kept in a supine position, with the head and shoulders elevated 15° to 20° above the rest of the body. They were maintained in this position for at least ½ hour after the solution had been introduced. The animals were allowed water but no food for the 18 hours preceding the experiment.

The amount of solution introduced into the lungs was carefully controlled, being set at 2 cc. per lb. of body weight. Except in rabbits, no difficulties were encountered in using this amount of fluid if about 5 minutes were allowed for it to run into the lung.

The animals were sacrificed from 15 minutes to 72 hours after the introduction of the fluid. Nembutal administered intravenously was used to stop the respirations. The abdomen was opened and the abdominal aorta cut prior to opening the chest and removing the lungs. The lungs were examined for gross changes and sections were taken from the more involved portions and placed in 10 per cent formalin. Histologic preparations were studied after staining with hematoxylin and eosin.

Cultures were made from the involved portions of the affected lobes of all animals. Using sterile precautions, a section of involved lung about 1 cc. in size was streaked across a blood agar plate and then ground in a mortar with about 4 cc. of sterile broth and carborundum. Two-tenths cc. of the ground mixture was transferred to each of the following: a blood agar plate, beef heart broth, and deep meat infusion broth. Blood cultures were obtained from the heart's blood at the time of sacrifice in some animals.

Rectal temperatures were obtained on many animals before and after the experiments and the animals were closely watched following the introduction of fluid into their lungs to note their reactions.

Results

The results of the experiments on dogs are given in Table I.

Within 1 hour after the introduction of normal saline solution into their lungs, 4 dogs (118 to 121) were sacrificed. Their lower lobes were heavy and distended, and mottled blue to purple. The cut surfaces were wet, and abundant, white, frothy material exuded from the bronchi, although sections taken from the involved portions of the lower lobes did not sink in the 10 per cent formalin used as a fixative. On histologic examination slight change only was seen. Congestion was minimal and there was only a rare polymorphonuclear leukocyte in the bronchi or alveoli. There was no pink-staining material in the alveoli or hemorrhage, as in dogs allowed to survive 6 hours or more.

Nineteen dogs were sacrificed 6 to 7 hours after the introduction of solution into their lungs. Studies were made on the possible effect of various anesthetics, solutions, and routes of administration of the solutions in this larger group. The gross appearance of their lungs, while presenting some individual differences, was in general fairly uniform. Portions of the lower and middle lobes in most instances appeared enlarged and dark red to purple. On section, fluid exuded from the cut surface and the bronchi contained white, frothy material. Only in a few instances was consolidation so extensive that portions of the lung sank in the fixative.

The histologic findings varied considerably in this group. In all of the lungs, polymorphonuclear leukocytes were found in the bronchi and alveoli. The degree of polymorphonuclear exudate in the bronchi and alveoli was graded in each instance on a scale ranging from 1 plus to 4 plus and the results are incorporated in Table I under the heading of "polymorphonuclear leukocytic exudate." A grade of 1 plus was assigned if the lungs had only an occasional polymorphonuclear leukocyte in the alveoli, as in dogs 137, 139, 149, and 150. If the alveoli and

TABLE I

Bacteriologic and Pathologic Findings on Dogs Receiving Sterile Normal Saline Solution, Isotonic Ringer's Lactate Solution, and Distilled Water by Bronchoscope or Intratracheally

Dog	Solution	Route administered	Duration of experiment	Anesthetic	Cultures				Polymorphonuclear leukocytic exudate
					Lung smear	Ground lung			
					Blood-agar plate	Blood-agar plate	Beef heart broth	Deep meat infusion broth	
121	Saline	Bronchoscopically	15 min.	Nembutal and morphine sulfate			Gram-negative rods	Staphylococcus*	+
120	Saline	Bronchoscopically	30 min.	Nembutal and morphine sulfate	Negative	Negative	Staphylococcus*	Staphylococcus*	o
118	Saline	Bronchoscopically	30 min.	Nembutal and morphine sulfate	Negative	Negative	*H. influenzae canis*	Negative	+
119	Saline	Bronchoscopically	60 min.	Nembutal and morphine sulfate	*B. bronchisepticus*	*B. bronchisepticus*	Staphylococcus*	*B. bronchisepticus*	±
106	Saline	Bronchoscopically	6 hrs.	Nembutal and morphine sulfate	Negative	Negative	Negative	Negative	++++
122	Saline	Bronchoscopically	7 hrs.	Nembutal and morphine sulfate	Negative	Negative	Negative	*B. bronchisepticus*	++++
123	Saline	Bronchoscopically	6 hrs.	Nembutal and morphine sulfate	Negative	Negative	*B. bronchisepticus*	Negative	++++
134	Saline	Intratracheally	6 hrs.	Nembutal and morphine sulfate	Staphylococcus*	Negative	*B. bronchisepticus*	*B. bronchisepticus*	++++
135	Saline	Intratracheally	6 hrs.	Nembutal and morphine sulfate	Negative	Negative		Staphylococcus*	++++

136	Saline	Intratracheally	6 hrs.	Novocaine	*B. bronchisepticus*	*B. bronchisepticus*	*B. bronchisepticus*	*B. bronchisepticus*	++
137	Saline	Intratracheally	6 hrs.	Novocaine	Negative	Negative	Gram-positive rods	Gram-positive rods	+
138	Saline	Intratracheally	6 hrs.	Novocaine	*B. bronchisepticus*		*B. bronchisepticus*	*B. bronchisepticus*	++
139	Saline	Intratracheally	6 hrs.	Novocaine	*B. coli* and staphylococcus	*B. coli* and staphylococcus	*B. coli* and staphylococcus	*B. coli* and staphylococcus	+
140	Saline	Intratracheally	6 hrs.	Sodium pentothal, i.v.	Negative	Negative	Negative		++++
141	Saline	Intratracheally	6 hrs.	Sodium pentothal, i.v.	Negative	Negative	Negative		+++
145	Ringer's lactate	Intratracheally	6 hrs.	Sodium pentothal, i.v.					++
146	Ringer's lactate	Intratracheally	6 hrs.	Sodium pentothal, i.v.	*B. subtilis*	*B. subtilis*	*B. subtilis*	*B. subtilis*	+++
147	Distilled water	Intratracheally	6 hrs.	Sodium pentothal, i.v.	Negative	Negative	Gram-negative rods	Negative	++
148	Distilled water	Intratracheally	6 hrs.	Sodium pentothal, i.v.	Kurthia	Kurthia		Kurthia	++
151	Distilled water	Intratracheally	6 hrs.	Sodium pentothal, i.v.	*Pseudomonas fluorescens*	*Pseudomonas fluorescens*	*Pseudomonas fluorescens*	*Pseudomonas fluorescens*	++++
152	Distilled water	Intratracheally	6 hrs.	Sodium pentothal, i.v.	Negative	Negative	Negative	Negative	++

TABLE I (*Cont'd.*)

Dog	Solution	Route administered	Duration of experiment	Anesthetic	Cultures				Polymorphonuclear leukocytic exudate
					Lung smear	Ground lung			
					Blood-agar plate	Blood-agar plate	Beef heart broth	Deep meat infusion broth	
149	Saline	Intratracheally	6 hrs.	Sodium pentothal, i.v.	Negative	Negative	Negative	Negative	+
150	Saline	Intratracheally	6 hrs.	Sodium pentothal, i.v.	Negative	Negative	Negative	Gram-negative rods	+
153	None		6 hrs.	Nembutal	Negative	Negative	Negative	Negative	o
154	None		6 hrs.	Nembutal	Negative	Negative	Negative	Negative	o
132	Saline	Intratracheally	17 hrs.	Nembutal	Negative	Negative	Negative	Staphylococcus	o
133	Saline	Intratracheally	18 hrs.	Nembutal	Negative	Negative	Negative	Negative	+
110	Saline	Bronchoscopically	24 hrs.	Nembutal	Negative	Negative	Negative	Gram-negative rods	++
109	Saline	Bronchoscopically	72 hrs.	Nembutal	Negative	Negative	Negative	Negative	+

* Species not determined.

bronchi were packed with polymorphonuclear leukocytes, the condition was graded 4 plus, as was true of dogs 106, 122, 123, 134, 135, 140, and 151. If only patches of alveoli were filled with polymorphonuclear leukocytes, the grade was 2 plus or 3 plus depending on the extent of pulmonary involvement. Other changes were seen in histologic sections. Congestion of the capillaries sometimes was marked but appeared to have no relation to the number of polymorphonuclear leukocytes. Hemorrhage was observed in some sections in both bronchi and alveoli. Some dogs, particularly those with large numbers of polymorphonuclear leukocytes in the alveoli and bronchi, showed considerable homogeneous, pink-staining material, interpreted as serum protein, in the alveoli. There was no necrosis, but a minor degree of cellular infiltration was noted in the walls of the bronchi and bronchioles, and the columnar cells lining the bronchi occasionally were detached.

The gross appearance of the lungs was not always in close correlation with the microscopic findings. The lungs of dog 149 were distended and heavy in both lower lobes and had a meaty appearance on section, with abundant frothy material exuding from the bronchi, but received a grade of only 1 plus on microscopic examination. In contrast, dog 148 had minimal gross evidence of consolidation, but had a 2 plus polymorphonuclear exudate.

There appeared to be some relation between the degree of polymorphonuclear exudate in this group of 19 dogs and the method of anesthesia. The least cellular exudate was observed in the 4 dogs in which only local anesthesia with novocaine had been used (dogs 136 to 139), while the greatest amount of exudate was seen consistently in the 5 dogs receiving a combination of nembutal and morphine (dogs 106, 122, 123, 134, and 135). These dogs were frequently semi-stuporous and unable to get on their feet 6 hours after the introduction of the fluid into their lungs, while the dogs which were given only novocaine were up and alert as soon as they were removed from the table. Intermediate in effect was sodium pentothal, which was used on the remaining 10 dogs. Most of these animals were able to be up when they were removed from the operating table, ½ hour after the introduction of the solutions. The polymorphonuclear exudate also appeared to be intermediate in amount between those of the other two groups of dogs. Two of the dogs receiving sodium pentothal (140 and 151) had a 4 plus response, 2 others (149 and 150) had a 1 plus reaction, and the remaining 6 were graded as either 2 or 3 plus (141, 145, 146, 147, 148, and 152). To determine whether or not a general anesthetic had any direct effect on the polymorphonuclear response, 2 dogs (153 and 154) were injected with nem-

butal in an amount to produce surgical anesthesia and sacrificed 6 hours later. There were no changes in their lungs, either grossly or microscopically.

The effect of the anesthetic agent probably is only one of the variables in the polymorphonuclear response. The slight response in the dogs under local anesthesia might have been related to the very active cough reflex which was present during the introduction of saline solution into the lungs and which could have reduced the total amount of solution reaching the alveoli. The cough reflex was completely abolished in dogs under general anesthesia and these animals probably received the full amount of saline solution introduced.

The type of solution introduced into the lungs of the dogs apparently did not affect the degree of polymorphonuclear response, since tissue sections showed the same variations regardless of whether saline solution, Ringer's lactate solution, or distilled water was employed.

There appeared to be no relationship between the polymorphonuclear response and the results of the lung cultures. Dogs with negative cultures, *e.g.*, 140 and 141, had a marked polymorphonuclear exudation and dogs with positive cultures, *e.g.*, 139, had only a slight response. It is doubtful whether all of the organisms recovered were pathogenic, particularly such bacteria as Kurthia in dog 148, *Bacillus subtilis* in dog 146, and *B. bronchisepticus* in dogs 122, 123, 136, and 138. Furthermore, even if the above organisms were pathogenic, they must have been relatively few, since they were recovered only on incubation in broth cultures and rarely on the cultures made on blood-agar plates. The relative unimportance of such bacteria as might have been swept into the lung by the solutions is attested by failure to recognize bacteria, either singly or in clumps, in microscopic sections.

Four dogs were sacrificed from 17 to 72 hours after the saline solution was introduced into their lungs (dogs 109, 110, 132, and 133). The extent of the polymorphonuclear reaction in the alveoli was diminished in this group as compared to those examined at 6 hours. Three of the dogs (109, 132, and 133) had either no polymorphonuclear leukocytes in the alveoli and bronchi, or only a 1 plus reaction. However, all 3 showed marked capillary congestion and thickening of the alveolar walls. Dog 110 had a 2 plus polymorphonuclear reaction at 24 hours, associated with edema, round cell infiltration, and an apparent thickening of the interstitial framework.

None of the dogs receiving saline solution into their lungs appeared to have any toxemia following the experimental procedure. On 11 of the dogs listed in Table I rectal temperatures were taken immediately

before the experiments and again 6 hours afterwards. None of these had a higher temperature 6 hours after receiving saline solution than the upper range of body temperature (38° to 40°C.) found before the experiment was begun.

The results of gross and microscopic studies of the lungs of rabbits given normal saline solution intratracheally under nembutal anesthesia (Table II) were strikingly different from those obtained in the dogs sacrificed at comparable periods following the experimental procedure. At most, the lower lobes of the rabbits were darker and more mottled than the upper lobes, which were spared. Congestion appeared to be present, but the lower lobes were not distended as in the dogs. The cut surfaces were not wet and practically no white froth was seen in the bronchi. The microscopic studies revealed far less change than was seen in the dogs' lungs. Rarely were granulocytes seen in the bronchi or alveoli. The greatest polymorphonuclear reaction was seen in rabbits 7, 9, and 10, and in them no more than a 1 plus response was observed. In their lungs the polymorphonuclear leukocytes were found in collapsed alveoli and were associated with large mononuclear cells. Thickening of the interstitial framework was present. There rarely was any hemorrhage into the alveoli and then only a few red blood cells were seen outside the capillaries. The pink-staining material so commonly seen in the bronchi and alveoli of the dogs was almost completely absent from the lungs of the rabbits. The alveolar walls were thickened, the alveoli partly collapsed, and the capillaries filled with red blood cells and granulocytes in all of the rabbits examined. These changes were most noticeable 6 to 24 hours after the solution was introduced, but had not entirely disappeared at 48 and 72 hours.

Cultures made from the lungs of the rabbits were negative, with one exception. Rabbit 9 had staphylococci on the blood-agar plate streaked with lung tissue, but all remaining cultures from the lungs of this rabbit were sterile.

Comment

Considerable experimental evidence has been gathered to show that the lung reacts to irritating substances by an exudation of polymorphonuclear leukocytes into the alveoli and bronchi.[1,3,4] In this respect normal saline solution has not been considered particularly irritating, largely as the result of the investigations of Winternitz.[1] Winternitz, however, studied the effect of normal saline in the lungs of dogs at a time when the polymorphonuclear reaction would not be expected to be maximum, according to the results obtained in the present study. He sacrificed dogs (1) within an hour of the injection of saline solution, (2) after

TABLE II

Bacteriologic and Pathologic Findings on Rabbits Receiving Normal Saline Solution Intratracheally

Rabbit	Solution	Duration of experiment	Anesthetic	Cultures				Polymorphonuclear leukocytic exudate
				Lung smear	Ground lung			
				Blood-agar plate	Blood-agar plate	Beef heart broth	Deep meat infusion broth	
12	Saline	3 hrs.	Nembutal	Negative	Negative			o
11	Saline	6 hrs.	Nembutal	Negative	Negative			o
9	Saline	6 hrs.	Nembutal	Staphylococcus	Negative	Negative	Negative	+
5	Saline	7 hrs.	Nembutal	Negative				o
7	Saline	20 hrs.	Nembutal	Negative	Negative		Negative	+
8	Saline	20 hrs.	Nembutal	Negative	Negative	Negative	Negative	o
10	Saline	24 hrs.	Nembutal	Negative	Negative	Negative		+
13	Saline	48 hrs.	Nembutal	Negative	Negative	Negative		o
14	Saline	72 hrs.	Nembutal	Negative	Negative	Negative		o

recovery from ether, and (3) 18 hours or more after the introduction of saline solution into the lungs, thus missing the more marked polymorphonuclear reaction which comes about 6 hours after introduction of the fluid. Our results parallel his in that we found a negligible reaction in the first hour and a subsiding response from 18 to 72 hours after the saline solution was given. The reaction continues to subside, according to Winternitz' investigations in dogs, as he found only minimal changes on the fourth day and entirely normal lungs on the tenth day.

The present experiments were designed to eliminate, so far as possible, factors which in themselves might lead to an exudative response in the alveoli, such as infection and aspiration. Bacteria were recovered from some of the lungs of dogs, but almost wholly in the broth cultures of ground lung in which one organism would be sufficient to give a positive culture. Bacteria were seldom recovered on solid media, thereby providing a general index of the infrequency with which organisms reached the alveoli. Furthermore, there were 2 dogs, 140 and 141, in which all of the cultures were negative and yet the exudate of polymorphonuclear leukocytes was 4 and 3 plus, respectively. It is also believed that aspiration of saliva and mucus from the nose and throat could not have been a responsible factor in the production of the polymorphonuclear response in the lungs of the dogs, since positive lung cultures and a more diversified flora in individual dogs would undoubtedly have been found more frequently if such had been the case.

The pathologic picture seen in the lungs of dogs in the present study resembles that described under a variety of circumstances. According to Robertson,[4] the exudation of blood cells, principally polymorphonuclear leukocytes, along with large numbers of red blood cells and plasma, as seen in the dogs sacrificed at 6 hours in the experiments reported here, characterizes the initial response to highly irritating living and nonliving materials. It is the same response as is seen in the early stages of pneumococcal pneumonia, "terminal" pneumonia, pneumonia seen in pulmonary passive congestion, and in shock [5] and postoperative pneumonitis.[6] The results of the present experiments on dogs indicate that the polymorphonuclear response in the lung is readily elicited in certain species even when relatively bland substances are introduced. The response of the lungs to solutions which approximate the concentration of electrolytes in the plasma indicates that some modification of current views may be necessary regarding the degree of irritation needed to produce polymorphonuclear exudation.

The minimal changes in the lungs of rabbits following the introduction of saline solution are in contrast to the marked polymorphonuclear

reaction seen in dogs. No explanation for the wide differences observed in the two species can be given. Saline solution is not the only substance, however, that has produced a different response in the lungs of these two species. The injection of virulent pneumococci intratracheally in rabbits in order to produce lobar pneumonia has resulted in a succession of almost complete failures,[7] whereas the injection in dogs has met with a high degree of success.[8] The relative difficulty encountered in producing lobar pneumonia in rabbits has never been satisfactorily explained. The lung of the dog appears to be more nearly like that of man in its response to virulent pneumococci. This similarity favors the hypothesis that the reaction of the human lung to saline solution, water, and Ringer's lactate solution would resemble that of the dog rather than that of the rabbit.

Conclusions

Sterile normal saline solution, Ringer's lactate solution, and distilled water produced a significant polymorphonuclear exudate in the alveoli and bronchi of dogs within 6 hours of their introduction into the lungs. The polymorphonuclear response was subsiding within 18 to 24 hours after the solutions had been introduced.

The degree of polymorphonuclear exudate appeared to be greater in dogs under deep and prolonged anesthesia than in those under light anesthesia. The manner of administration and the type of solution did not appear to affect the degree of polymorphonuclear exudate in the lungs of dogs.

Sterile normal saline solution introduced into the lungs of rabbits resulted in little or no polymorphonuclear exudate.

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MYOCARDITIS IN VITAMIN E-DEFICIENT RABBITS *

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Vitamin E deficiency in the rabbit manifests itself in muscular weakness which, if the animal survives long enough, terminates in extensive paralysis. Pathologically, the voluntary muscles show what has long been recognized in man as Zenker's degeneration. Most of the earlier publications on this "nutritional muscular dystrophy" either make no reference to myocardial studies or contain the statement that the heart is unaffected.[1-3] On the other hand, some observations have been published indicating that myocardial damage also occurs. Madsen,[4] in 1936, described and illustrated degenerative and infiltrative lesions in the myocardium of dystrophic rabbits, but drew no conclusions. Houchin and Smith,[5] the first to claim a causal relationship between tocopherol deficiency and myocardial damage, demonstrated that slices of heart muscle from dystrophic rabbits, like slices of skeletal muscle, exhibit an increased oxygen consumption. They also showed in the intact deficient rabbit an increased sensitivity to pitressin and a greater resistance to ouabain and digoxin. More recently, Gatz and Houchin [6,7] have reported electrocardiographic and histologic changes regarded by them as evidence of myocardial degeneration in E-deficient rabbits.

It is the purpose of this paper to report additional observations, both electrocardiographic and morphologic, on myocardial abnormalities in rabbits receiving E-deficient diets.

METHODS

Young male and female white rabbits weighing between 600 and 1300 gm., housed in individual cages on wire grids, were offered the following simplified E-deficient diets *ad libitum*. The two diets differ primarily in fat content. Animals gaining weight consumed 35 to 45 gm. of diet daily.

	Diet 1	Diet 2
Vitamin-free casein	15 per cent	15 per cent
Lard	20 per cent	10 per cent
Sucrose	59 per cent	70 per cent
Salt mixture †	6 per cent	5 per cent

Both diets were supplemented with equal quantities of vitamins.‡ Each

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† Formula of "salts 4" (Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B. Choline in the nutrition of chicks. *J. Biol. Chem.*, 1941, **138**, 459-466).

‡ Vitamins were added in the following quantities per 100 gm. of diet; Thiamine, 1 mg.; pyridoxine, 1.5 mg.; riboflavin, 2 mg.; calcium pantothenate, 3 mg.; nicotinic acid, 30 mg.; choline chloride, 300 mg.; vitamin A (afaxin), 1000 units; and vitamin D (drisdol), 100 units. All vitamins were supplied through the courtesy of Winthrop-Stearns, Inc., New York 13, N.Y.

animal also received 0.5 cc. of a refined corn oil (mazola) three times a week. For the control animals, 15 mg. of alpha-tocopherol were dissolved in each 0.5 cc. of corn oil. Roughage was supplied from time to time in the form of filter paper.

The electrocardiograms were taken with a portable clinical machine (cardiette) in the three conventional leads only. The animals were unanesthetized but blindfolded.

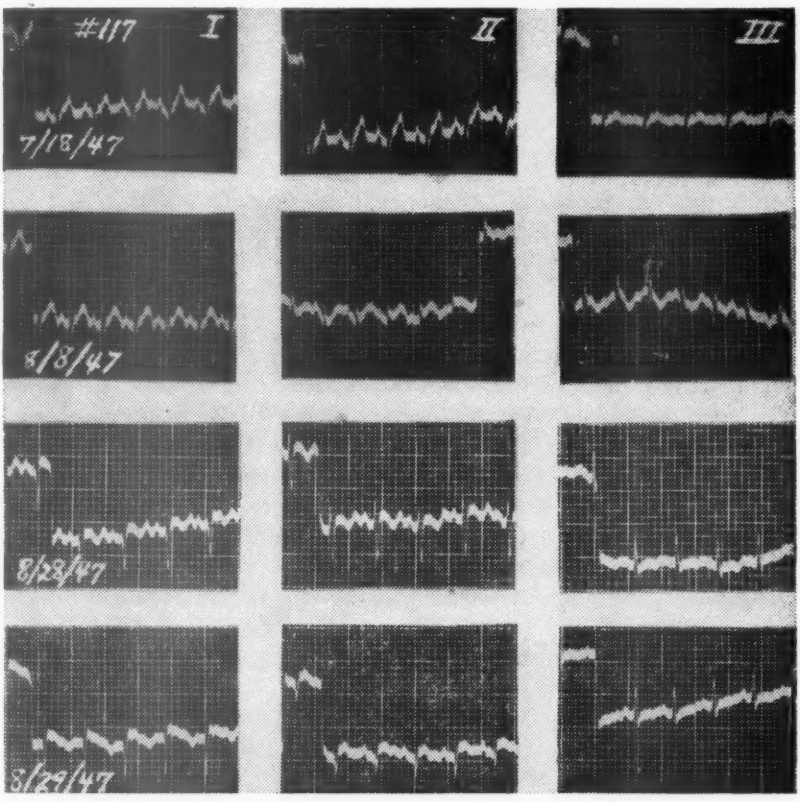
Results

Of 19 rabbits receiving the deficient diets, 17 survived long enough to develop clinical signs of muscular dystrophy; they comprise the material for this report. Twelve of these animals received diet 2; and 5, diet 1. All of the former group gained weight for about 4 weeks, then sickened, and were either sacrified or died, usually in the 7th week. The 5 rabbits on the higher fat diet failed to gain weight. They developed signs of dystrophy and died, on the average, 1 week earlier than those on the lower fat diet.

There were 6 control rabbits, 3 on each diet. None developed muscular dystrophy. Four of them continued to gain weight until they were sacrificed 6 to 8 weeks later. The other 2, during the terminal weeks, showed a significant loss of weight, presumably the result of severe coccidial cirrhosis as demonstrated at necropsy.

Electrocardiograms were taken only on rabbits receiving diet 2. Seven of the deficient animals had two or more tracings. In each case a control tracing was taken before the animal was placed on the diet and another was taken immediately prior to sacrifice. The later tracings of 5 of these animals showed changes that are interpreted as definite abnormalities. These include elevation of the S-T segment and inversion of T waves in lead II (Text-Figs. 1 and 2). These changes did not develop until the latter part of the period of observation, when muscular dystrophy was already severe. They did not occur in any of the control animals receiving the same diet supplemented with alpha-tocopherol; nor were they ever observed in a series of 23 "normal" rabbits.[8]

All 17 rabbits on the deficient diets showed the typical gross and microscopic changes in their skeletal muscle described by Goettsch and Pappenheimer [1] in nutritional muscular dystrophy. In addition, 15 of them showed foci of necrosis accompanied by an inflammatory reaction in the myocardium (Figs. 1 to 3). The sequence of morphologic events in the cardiac muscle resembled that in the voluntary muscle and appeared to be as follows: A coagulative necrosis of sarcoplasm with loss of striations; pyknosis and later karyorrhexis of muscle nuclei; and an

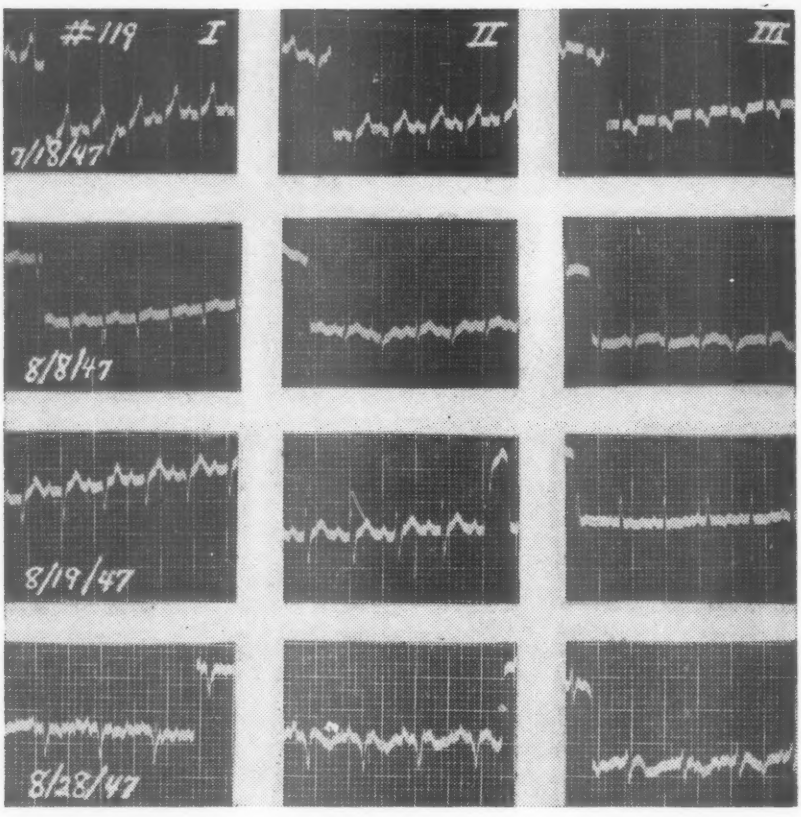


Text-Figure 1. Serial, conventional lead, electrocardiograms in vitamin E-deficient rabbit 117. Control tracing was taken on July 18, 1947. Reversal of T 3 on August 8 is a normal variation. Elevation of S-T 1 and 2 on August 28 and inverted T 1 and 2 on August 29 are abnormal findings. The animal was sacrificed the same day and necropsy revealed grossly visible areas of necrosis in the wall of the left ventricle.

inflammatory reaction characterized by edema, small hemorrhages, and the infiltration of monocytes and polymorphonuclear neutrophils in varying proportions.* In animals in which lesions of the skeletal muscle showed calcification, there was usually calcification of necrotic cardiac muscle as well. There was no clear evidence of regeneration of cardiac muscle. Myocarditis was consistently more extensive in the 5 animals receiving the higher fat diet.

The most frequently involved sites were the posterior walls of the

* In several instances sections were examined with the Gram stain. Even in animals that had lain dead overnight at room temperature, no organisms were found in the heart lesions.



Text-Figure 2. Serial electrocardiograms in vitamin E-deficient rabbit 119. Control tracing was taken on July 18, 1947. Tracing of August 8 shows low voltage but is not otherwise remarkable. On August 19, P-R was prolonged to 0.09 seconds in lead I (upper limit of normal), but this increase is partly explained by cardiac slowing. On August 28, inversion of T 1, 2, and 3 can be seen despite marked somatic tremor. This is an abnormal finding. The animal was found dead the following morning. Necropsy showed grossly evident lesions in both ventricles, ascites, hydrothorax, and passive congestion of viscera.

ventricles and the papillary muscles. In several animals the auricular muscle also was involved. When severe, the lesions were readily visible grossly as rather well circumscribed, gray areas. In one animal (119), the changes were more diffuse and were accompanied by the usual signs of congestive heart failure.

From the control animals the same number of sections were cut as from the deficient group. In the wall of the left ventricle of one rabbit there was a solitary lesion characterized by basophilia of the sarcoplasm

with loss of striations but without inflammatory reaction. In the other 5 animals no myocardial lesions were observed.

In addition to these changes in voluntary and cardiac muscle, 3 of the 5 animals receiving deficient diet no. 1 showed foci of necrosis and polymorphonuclear infiltration in the smooth muscle of the stomach and in scattered arterioles, particularly of the lung. These findings were most severe, even grossly obvious, in the gastric fundus. Although never present in the controls, these lesions were not encountered frequently enough to warrant relating them to tocopherol deficiency.

Discussion

That the myocarditis observed in these rabbits was the direct result of the tocopherol deficiency is evidenced primarily by the frequency of its occurrence in the deficient group (88 per cent) compared with its absence in the control group. On the other hand, other investigators have observed no heart lesions in severely dystrophic rabbits. The possibility cannot be excluded that these lesions are coincidental findings facilitated rather than caused by the vitamin deficiency. Because myocarditis of unknown etiology has been observed in "normal" rabbits, it should be emphasized that the animals in this series were young and that the lesions were all acute.

Although the lesions in the heart were neither so extensive nor so diffuse as those in the skeletal muscle, the differences were essentially quantitative. It has been shown that in rats on a low tocopherol intake the heart contains proportionately twice as much of the vitamin as does the skeletal muscle.[9] A comparable distribution in the rabbit might explain not only the difference in the extent of the lesions but also the delay in the appearance of electrocardiographic changes until the muscular dystrophy was well advanced. Return of these changes to normal by the addition of tocopherol was not attempted because at the stage in which they appeared the animals were considered beyond recovery.

The physiologic rôle of vitamin E remains unknown. Although its deficiency in various animals produces strikingly similar lesions in skeletal muscle, there remain peculiar species specificities. These include encephalomalacia in the chick,[10] irreparable degeneration of germinal epithelium in the rat,[11] and necrosis of smooth muscle in the gizzard of the turkey.[12] Mason and Emmel [13] observed myocardial lesions of a chronic nature in rats maintained for over 1 year on an E-deficient diet. The only other species in which myocardial damage has been claimed in this deficiency is the cow.[14] Further generalizations are not warranted.

Summary

Among 17 rabbits that developed severe muscular dystrophy on vitamin E-deficient diets, 15 (88 per cent) showed foci of acute myocarditis at necropsy. In some cases there were abnormal electrocardiographic changes as well. Animals receiving the same diets supplemented with alpha-tocopherol failed to show these changes.

The sequence of pathologic events in cardiac muscle resembled the changes in voluntary muscle, although the lesions in the former were less diffuse.

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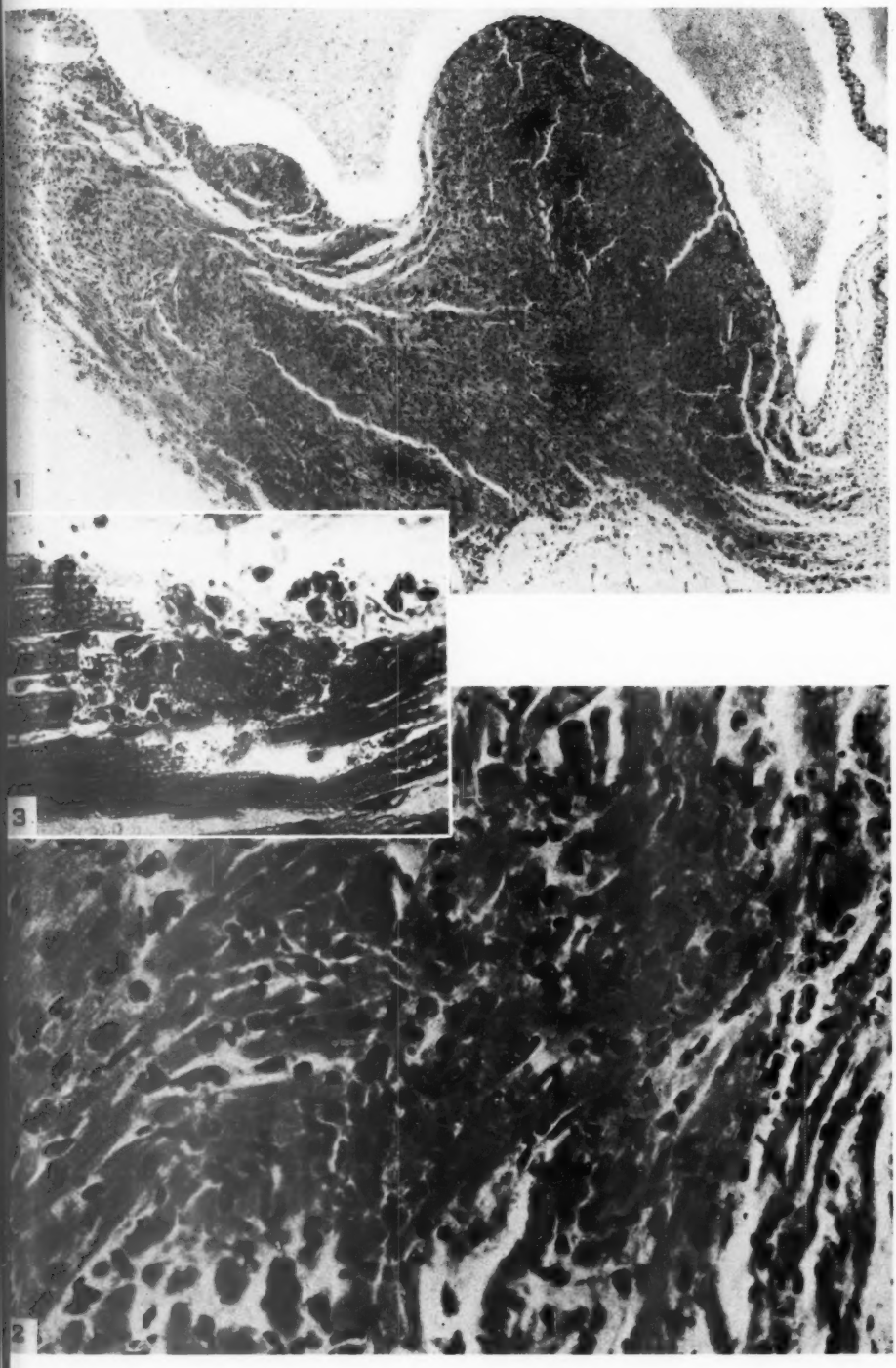
Description of Plate

Plate 35

Fig. 1. Rabbit A3. Section of the wall of the right ventricle and pulmonic cusp. This animal became severely dystrophic and was sacrificed after 4½ weeks on diet 1. There had been no weight loss. × 100.

Fig. 2. Rabbit A2. A rather typical lesion in the ventricular wall, showing necrosis and fragmentation of sarcoplasm with complete loss of normal structure and infiltration of inflammatory cells. × 800.

Fig. 3. Rabbit 129. Fragmentation of a single myocardial fiber and polymorphonuclear infiltration. × 800.



Bragdon and Levine Myocarditis in Vitamin E-Deficient Rabbits

FACTORS INFLUENCING COLLAGEN CONTENT IN EXPERIMENTAL CIRRHOSIS *

THOMAS G. MORRIONE, M.D.†

(From the Department of Pathology, University of Vermont, College of Medicine, Burlington, Vt.)

In a previous study,[1] the increases in collagen which occur in experimental cirrhosis due to carbon tetrachloride and *p*-dimethylaminoazobenzene were studied by chemical means. It was shown, also, that partial or complete disappearance of collagen occurs after the administration of these hepatotoxic substances is stopped. The present observations concern the rate at which collagen is deposited and resorbed in experimental cirrhosis, as well as factors which influence these changes.

METHODS

A total of 252 male albino rats, weighing 180 to to 220 gm. each, were employed. Hepatic cirrhosis was produced in 234. The rats, in groups of approximately 40, were placed in an air-tight, glass-topped chamber. Carbon tetrachloride vapor was introduced into the chamber until the animals became semi-comatose. They were maintained in this state for several minutes. The concentration of the vapor was then raised until the animals became unconscious. The glass top was then quickly removed and the interior of the chamber was ventilated by vigorous fanning. The rats regained consciousness rapidly. This procedure was repeated three times during the course of 20 minutes. Exposure to carbon tetrachloride was repeated every other day for a period of 35 days. Groups of animals were sacrificed at 5-day intervals, and collagen determinations were performed on 2 to 3 gm. samples of their livers in order to determine the rate of collagen deposition. On the 36th day, the remaining rats, selected at random, were divided into 7 groups. One group was left on a normal diet; from these, animals were periodically sacrificed to ascertain the rate of collagen resorption. Five groups of 20 rats each were placed on special diets. Ligation of the portal vein was performed on the last group with cirrhosis according to the method of Whitaker.[2] Six normal control rats were sacrificed to determine the normal value of hepatic collagen, and ligations of the portal vein were performed on 12 additional normal rats.

* Aided by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

Read by title at the Forty-Fifth Annual Meeting of The American Association of Pathologists and Bacteriologists, Philadelphia, March 12 and 13, 1948.

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METHODS

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The method of Lowry, Gilligan, and Katersky [3] was modified as follows, in order to determine quantitatively the collagen content of liver: Pound and grind the sample thoroughly. Transfer to a round-bottomed, 50 cc. pyrex centrifuge tube with 40 cc. of distilled water. Centrifuge and discard the supernatant liquid. Add 40 cc. of distilled water and allow to stand for 5 minutes. Centrifuge and discard the supernatant liquid. Add 40 cc. of 0.1 normal sodium hydroxide, stir, and allow to stand for 5 minutes. Centrifuge and remove the supernatant liquid. Add 40 cc. of 0.1 normal sodium hydroxide, stir, and let stand overnight at room temperature. Stir, centrifuge, and remove the supernatant liquid. Add 40 cc. of 0.1 normal sodium hydroxide and let stand 1 hour with frequent stirring. Centrifuge and remove the supernatant liquid. Add 40 cc. of distilled water and 1 drop of 0.1 per cent phenol red indicator. Adjust color to faint pink with 0.1 normal hydrochloric acid, allowing time for diffusion of alkali from the tissue fragments. Centrifuge, and discard the supernatant liquid. Add 40 cc. of a mixture of 3 parts of 95 per cent alcohol and 1 part absolute ether. Let stand for 20 minutes with occasional stirring; centrifuge and remove the supernatant liquid. Add 40 cc. of absolute ether, mix, and let stand for 10 minutes. Centrifuge and remove the supernatant liquid. Dry to constant weight in an oven at 100° C. (2 to 4 hours), and add 40 cc. of distilled water. Stopper the tubes with nonabsorbent cotton and autoclave for 4 hours, at 50 lbs. pressure. Cool, centrifuge, discard the supernatant liquid, and dry to constant weight. The weight before autoclaving minus the weight after autoclaving represents the weight of collagen in the sample, since the collagen is converted by the autoclaving into soluble gelatin.

All animals were autopsied and sections of the livers were stained with hematoxylin and eosin, Laidlaw's reticulum stain counterstained with van Gieson's mixture, and Masson's trichrome stain.

Results

The increases in collagen observed with progressive cirrhosis are shown in Table I and in Text-Figure 1. Elevated values were noted in both the percentage of collagen and in the total hepatic collagen. The deposition of collagen shown in Text-Figure 1 follows a curve of exponential type. The early lag phase most probably represents the early proliferation of fibroblasts, which progresses in a 1, 2, 4, 8, 16-manner. Collagen formation accordingly occurs at a relatively greater rate as geometric fibroblastic multiplication proceeds.

The lower normal value for collagen content, *i.e.*, 0.16 per cent (Table I), as compared to 0.23 per cent found in a previous study, may be

accounted for by the modified procedure which was employed in the present experiments. The preliminary extraction with water, however, is advisable with liver in order to avoid the markedly viscous solution which results when dilute alkali is employed for the first extraction.

An explanation for the irregularities in the reversal phase shown in Text-Figure 1 may be found in the fact that fewer animals (25 rats) were employed in this phase as compared to the 57 which were used to determine the rate of collagen deposition. The incompleteness of the

TABLE I

Collagen Content in Hepatic Cirrhosis Due to Carbon Tetrachloride

Days	Rat no.	Weight of rat	Weight of liver	Collagen, wet weight	Total hepatic collagen	Average collagen, wet weight for the group
		gm.	*gm.*	*per cent*	*mg.*	*per cent*
Normal	1	182.0	10.7	0.24	25.7	
	2	188.8	10.6	0.14	14.8	
	3	204.0	12.5	0.12	15.0	
	4	207.1	12.1	0.12	14.5	
	5	224.5	13.5	0.21	28.4	
	6	227.3	13.4	0.10	13.4	0.16
5	7	182.1	9.2	0.21	19.3	
	8	188.1	10.5	0.19	19.9	
	9	192.3	10.4	0.18	18.7	
	10	203.1	10.1	0.15	15.2	
	11	212.8	11.3	0.16	18.1	0.18
10	12	145.5	10.2	0.19	19.6	
	13	173.1	13.2	0.11	14.5	
	14	190.7	11.7	0.18	21.1	
	15	205.4	11.2	0.20	22.4	
	16	207.0	10.6	0.29	30.7	
	17	216.3	15.1	0.33	49.8	0.22
15	18	174.9	9.4	0.27	25.4	
	19	180.4	12.3	0.34	41.8	
	20	183.4	12.5	0.36	44.0	
	21	190.2	10.4	0.24	24.9	
	22	224.5	18.0	0.25	45.0	
	23	242.9	15.5	0.21	32.6	0.28
20	24	116.0	5.6	0.27	15.1	
	25	135.6	8.5	0.48	40.8	
	26	137.5	11.1	0.25	27.8	
	27	157.0	10.0	0.35	35.5	
	28	199.2	14.2	0.40	56.8	
	29	201.7	12.6	0.25	31.5	
	30	220.1	14.4	0.23	33.1	
	31	270.7	13.1	0.22	28.8	0.31
25	32	122.4	6.7	0.49	31.9	
	33	144.6	12.0	0.50	60.0	
	34	151.9	13.9	0.28	38.9	
	35	160.5	10.0	0.28	28.0	
	36	164.9	17.5	0.46	80.5	
	37	176.1	12.5	0.24	30.0	
	38	189.6	11.4	0.37	42.2	
	39	199.7	11.4	0.43	49.0	
	40	207.9	12.2	0.26	31.7	0.37

TABLE I (*Con't.*)

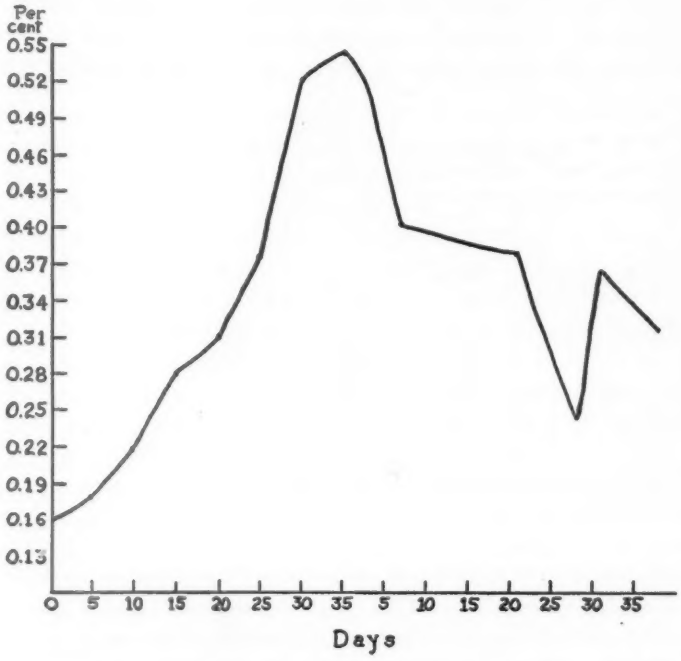
Days	Rat no.	Weight of rat	Weight of liver	Collagen, wet weight	Total hepatic collagen	Average collagen, wet weight for the group
		gm.	*gm.*	*per cent*	*mg.*	*per cent*
30	41	103.4	6.6	0.63	41.6	
	42	130.0	12.9	0.45	58.1	
	43	142.3	9.6	0.49	47.0	0.52
35	44	108.4	6.0	0.40	24.0	
	45	116.4	7.4	0.78	57.7	
	46	131.7	9.5	0.76	72.2	
	47	131.8	5.7	0.45	25.7	
	48	150.6	13.2	0.42	45.4	
	49	153.8	10.0	0.54	54.0	
	50	155.0	11.4	0.36	41.0	
	51	158.7	9.9	0.73	72.3	
	52	163.0	10.9	0.45	49.1	
	53	166.3	10.9	0.40	43.6	
	54	171.2	13.0	0.76	98.8	
	55	179.2	12.6	0.64	80.6	
	56	186.5	13.7	0.50	68.5	
	57	215.8	13.5	0.36	48.6	0.54
		Reversal of cirrhosis on normal diet*				
3†	58	87.3	5.2	0.62	32.2	
	59	116.1	5.7	0.83	47.3	
	60	132.1	11.0	0.35	38.5	
	61	132.6	9.8	0.30	29.4	
	62	156.2	12.1	0.70	84.7	
	63	159.7	8.9	0.31	27.6	
	64	193.6	12.3	0.44	54.1	0.51
7†	65	118.1	7.5	0.29	21.8	
	66	133.0	7.9	0.57	45.0	
	67	148.4	8.8	0.35	30.8	
	68	153.1	8.1	0.48	38.9	
	69	244.4	14.4	0.30	43.2	0.40
21†	70	123.3	7.0	0.31	21.7	
	71	195.3	10.7	0.45	48.2	
	72	211.3	10.0	0.39	39.0	
	73	233.1	11.1	0.35	38.9	0.38
28†	74	176.3	8.0	0.28	22.4	
	75	219.1	9.4	0.21	19.7	
	76	240.2	10.3	0.23	23.7	0.24
31†	77	185.8	7.7	0.28	21.6	
	78	241.6	9.8	0.44	43.1	0.36
38†	79	153.5	7.6	0.33	25.1	
	80	176.2	9.3	0.29	27.0	
	81	181.9	8.4	0.39	32.8	
	82	227.5	10.8	0.28	30.2	0.32

* Dog chow pellets.
† Days after last exposure to carbon tetrachloride.

reversal which took place is shown by the 0.32 per cent collagen content level at 38 days, as well as by microscopic evidence of persisting cirrhosis in these animals.

Reversal of Cirrhosis on Special Diets

One hundred rats, on carbon tetrachloride for 35 days, were placed on special diets the day after the carbon tetrachloride was stopped. Twenty animals were employed in each of 5 groups. The diets used are shown in Table II.



Text-Figure 1. Graphic representation of data presented in Table I. Alterations in collagen content in cirrhosis due to carbon tetrachloride. The drug was discontinued on the 35th day.

All diets were fed to the animals with cirrhosis for a period of 35 days. Decreases in hepatic collagen, determined chemically and confirmed by microscopic study, were observed with all five diets. The greatest decrease occurred with diet B, a low protein diet supplemented with methionine, choline, and cystine. The arithmetic mean of the hepatic collagen content after 35 days on diet B was 0.28 per cent, as compared to 0.47 per cent with diet D, a low protein, high fat diet. On diets A, C, and E, the average collagen content was 0.36, 0.34, and 0.36 per cent, respectively, after 35 days of reversal on these diets. These latter values are not significantly different from the level of 0.32 per cent which was

observed after 38 days' reversal on the normal diet (Table I). Microscopic evaluation of collagen content recorded as 1 to 4 plus closely paralleled the chemical findings.

Effect of Ligation of the Portal Vein on the Collagen Content of Normal Liver and Livers with Cirrhosis Due to Carbon Tetrachloride

Ligations of the portal vein were performed on 12 normal control rats. The animals were autopsied 34 to 42 days after the second stage of the operation. All showed adhesions between the liver and adjacent structures, including the intestine, omentum, diaphragm or anterior abdominal wall. The adhesions were marked in some, and minimal or slight in others. The average collagen content for this group was 0.30 per cent wet weight, as compared to the normal of 0.16 per cent. The average for hepatic collagen was 25 mg. per liver as compared to 18.6 mg. in normal livers. Microscopically, increased amounts of reticulum were visible (Fig. 7). Some of this increase was due to condensation of the normal stroma, since the livers in this group showed atrophy, their weights being less than those of normal controls and lower than the normal weight of the rat's liver according to Webster, Liljegren, and Zimmer.[4] The increase in the average total hepatic collagen following ligation of the portal vein, in spite of atrophy of the liver, indicates an absolute as well as a relative increase in collagen.

TABLE II

Composition of Diets Employed in Reversal of Cirrhosis Due to Carbon Tetrachloride

Ingredient	Diet A Low protein	Diet B* Low protein	Diet C High protein	Diet D Low protein, high fat	Diet E High carbohydrate, low fat
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Casein	4	4	56	4	18
Cornstarch	30	30	4	22	33
Dextrose	30	30	4	22	33
Crisco	22	22	22	38	2
Cod-liver oil	2	2	2	2	2
Salt mixture	4	4	4	4	4
Yeast powder no. 300†	8	8	8	8	8

* Supplemented with methionine, 500 mg.; choline, 125 mg.; and l-cystine, 312.5 mg. per 100 gm. of diet.

† Anheuser-Busch.

The portal vein was ligated in a group of 12 rats with hepatic cirrhosis, obtained from the same original large group of rats with cirrhosis due to carbon tetrachloride. The first stage of the operation was performed on the day after the carbon tetrachloride was discontinued, and the

second stage was done 3 days later. The rats were then autopsied in groups of 4 on the 16th, 23rd, and 36th day after the second operation. The average content of hepatic collagen in these groups was 0.35, 0.35, and 0.33 per cent, respectively. The only animal which showed no adhesions to the liver (Fig. 6) had the highest percentage of collagen of the entire group, 0.52 per cent. No correlation was apparent between the number or size of the adhesions and the amount of cirrhosis which persisted.

Discussion

Involutionary changes became apparent in the fibroblasts as reversal of the hepatic cirrhosis progressed (Fig. 2). Most of the fibroblasts were located in the fibrous trabeculae, and, as the latter became narrowed (Figs. 3 and 4), the cellular as well as the fibrillar elements became scarcer. Save for a gradual disappearance of reticular and collagenous fibers, no microscopic morphologic or staining peculiarities were seen in the connective tissue which was undergoing resorption. Occasional macrophages containing nuclear débris were identified in livers during the reversal phase, but no phagocytosis of fibrils or evidence of localized erosive action by phagocytes was observed.

The beneficial effect of supplemental methionine, choline, and cystine upon the reversal of cirrhosis on a low protein diet is most probably a result of the favorable influence which these substances exert upon the liver cells themselves, the cystine being beneficial only in small amounts to supplement a low protein intake.[5,6] An opposite and deleterious effect was seen when hepatic cells were damaged with a high fat, low protein diet, or when the portal vein was ligated. Although high fat, low protein diets produce hepatic cirrhosis, new deposition of fibrous tissue due to this diet does not occur during the short interval for which it was employed in the present study.[7] The persistence of cirrhosis following successful ligation of the portal vein is in accord with Mann's [8] demonstration that regeneration of the liver following partial hepatectomy is considerably impaired when no portal venous flow is present. It appears likely, then, that the disappearance of collagen from cirrhotic livers is closely related to, and is dependent upon, the functional state or regenerative activity of the liver cells themselves.

As previously noted,[1] the chemical values obtained by Lowry's method correlate best with microscopic evaluations which are based upon reticulum staining, rather than upon the trichrome method. This finding was confirmed in the present study, especially with regard to the increased reticulum which was observed following ligation of the portal vein. In these livers, the trichrome stain showed no appreciable deposit

of collagen, but parallel increases were seen in both the chemical values and in the amount of reticulum which was visible microscopically.

Summary and Conclusions

Hepatic cirrhosis was produced in a group of 234 rats by exposure to carbon tetrachloride vapors every other day for 35 days.

The increase in collagen, as determined chemically, followed a curve of exponential type. Decrease in the collagen content occurred after stopping the carbon tetrachloride.

Reversal of cirrhosis was very nearly complete, and equally so with a normal diet, or with a low protein diet supplemented with methionine, choline, and cystine. Recovery from cirrhosis was impaired by a high fat, low protein diet.

Ligation of the portal vein also retarded recovery from cirrhosis, unless hepatic adhesions were present. The latter favored reversal of the cirrhosis.

Resorption of collagen from livers with cirrhosis depends upon the functional state or regenerative activity of the hepatic cells themselves.

Comparison of microscopic and chemical observations indicates that the method of Lowry determines reticulum as well as collagen.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 36

FIG. 1. Active proliferation of fibroblasts and bile duct epithelium in hepatic cirrhosis due to exposure to carbon tetrachloride for 20 days. The larger cells of a bile duct at the lower left corner are distinguishable from the plump, irregular, ovoid, fibroblastic nuclei elsewhere. Hematoxylin and eosin stain. × 1000.

FIG. 2. Cirrhosis due to carbon tetrachloride 14 days after stopping the drug. The fibroblastic nuclei, in comparison to those seen in Figure 1, are shrunken and pyknotic. Hematoxylin and eosin stain. × 1000.

FIG. 3. Cirrhosis due to exposure to carbon tetrachloride for 30 days. Fibrillar collagen and reticulum form broad septa. Laidlaw's reticulum and van Gieson's stains. × 200.

FIG. 4. Reversal of cirrhosis 30 days after discontinuing carbon tetrachloride. There is considerable disappearance of collagen and reticulum, with marked narrowing of the connective tissue septa as compared to Figure 3. Laidlaw's reticulum and van Gieson's stains. × 200.


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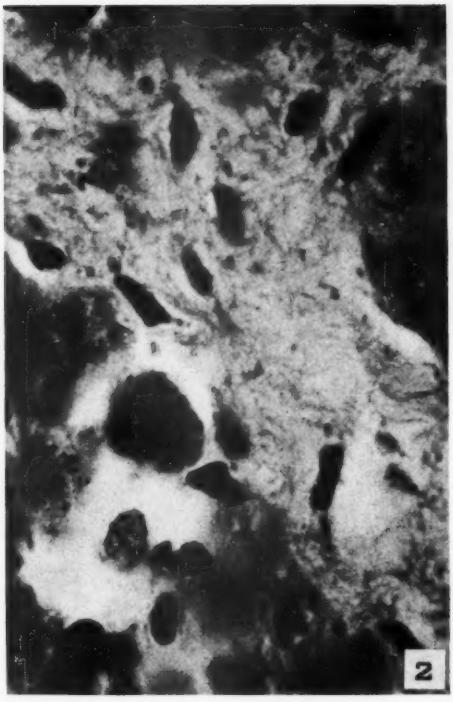
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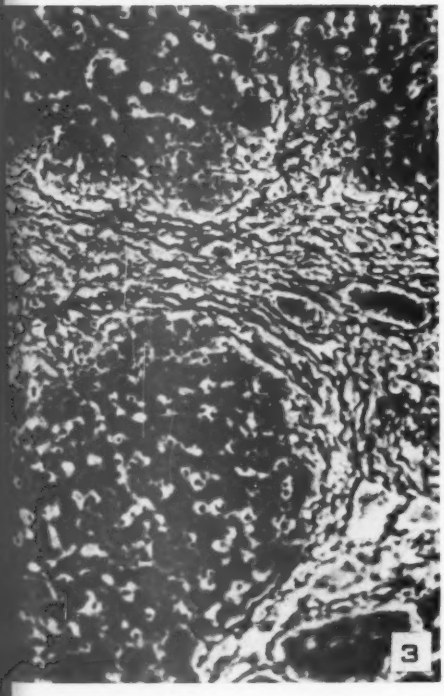
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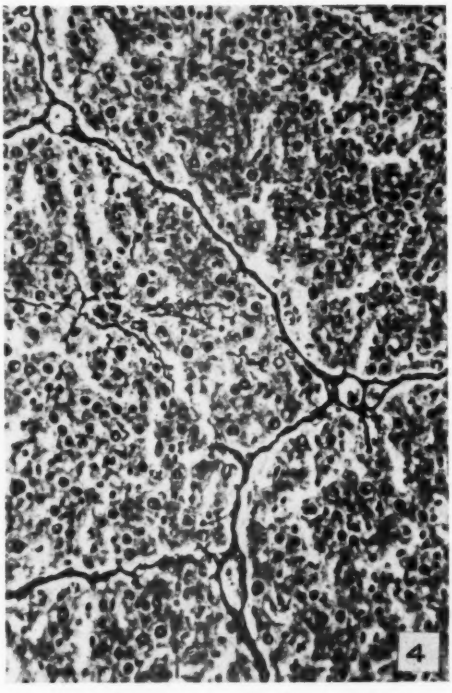
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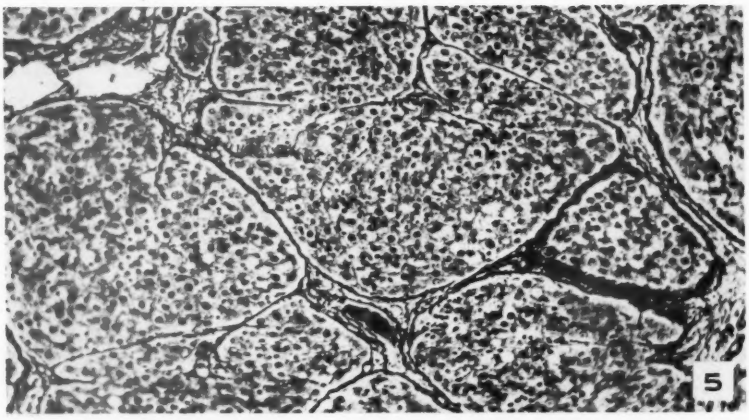
PLATE 37

FIG. 5. Persisting cirrhosis 38 days after stopping the administration of carbon tetrachloride. Animals were fed a low protein, high fat diet during the period of reversal. Collagen content was 0.68 per cent. Laidlaw's reticulum and van Gieson's stains. × 175.

FIG. 6. Failure of reversal of carbon tetrachloride cirrhosis in a rat with ligation of the portal vein. Collagen content was 0.52 per cent 23 days after stopping the drug. The portal vein was ligated after the cirrhosis had been produced. No hepatic lesions were present. Laidlaw's reticulum and van Gieson's stains. × 175.

FIG. 7. Increased reticulum 42 days following ligation of the portal vein in a normal rat. Collagen content was 0.35 per cent. Laidlaw's reticulum and van Gieson's stains. × 500.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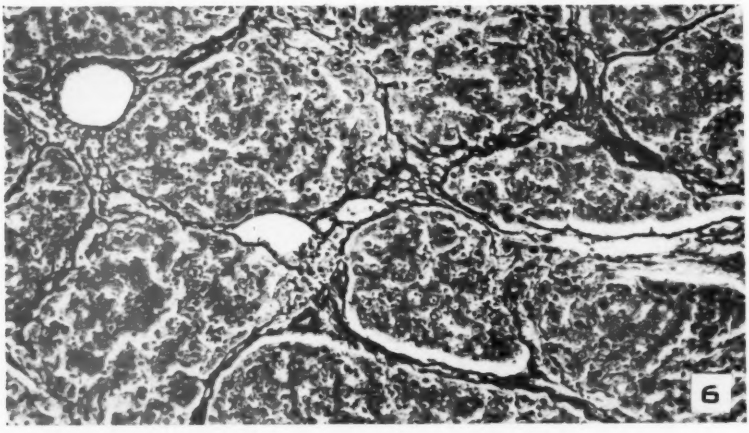
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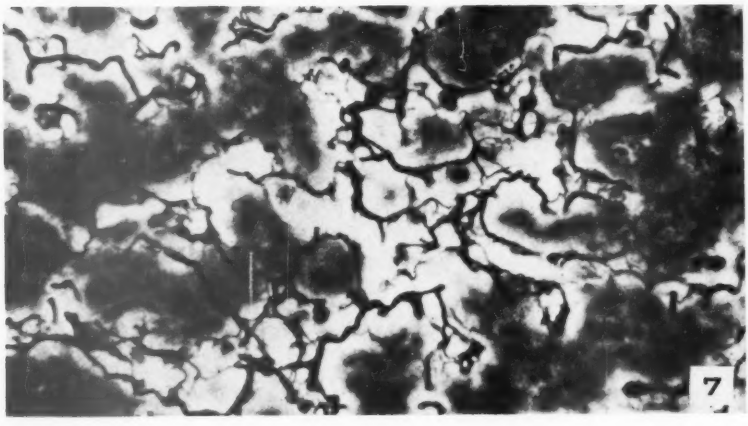
7

CUTANEOUS LEIOMYOMA OF GOLDFISH

I. Morphology and Growth in Tissue Culture *

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Approximately 3 to 4 per cent of the goldfish in a 3-acre urban pond have been found to bear single or multiple cutaneous tumors. The fish averaged 25 cm. in length; a study of their scales showed that the majority were about 5 years old. They appeared healthy, although an occasional specimen either with or without a tumor was infested with copepods of the species *Lernaea carassi* (Tidd). The pond was created 20 years ago by damming a small stream; at present most of the water is supplied by a spring. The fishes, including goldfish, sunfish, and minnows, are descendants of those originally used to stock the pond. Only the goldfish bore cutaneous neoplasms.†

Gross Examination

The descriptions of the gross and microscopic appearance of these tumors are based upon the study of material obtained from 14 goldfish (*Carassius auratus*) that were kept under observation in aquaria for periods ranging from 45 to 203 days. When small, the tumors were moderately firm and orange-yellow. They arose at the base of a scale, growing over and burying it as they increased in size; the free surfaces were covered by epidermis. As the neoplasms became larger they remained broadly sessile, were softer, and sometimes displayed several areas of cystic degeneration. The surface was smooth, but in the large growths it was sometimes faintly lobulated or even ulcerated. On section there was profuse bleeding; the cut surface was homogeneous and gray-pink.

The tumors occurred in widely scattered regions of the trunk as well as upon the head and all the fins. Although no area of predilection has been observed, three of the largest and most rapidly growing neoplasms arose on the trunk immediately behind the operculum (Fig. 1). Such large tumors usually were single, although some were accompanied by 2 or 3 small nodules on the trunk or fins. Multiple growths (5 to 10) usually were small, seldom over 15 mm. in greatest diameter (Fig. 2).

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† Permission to remove these fish from the pool on the grounds of The Cleveland Museum of Art was given by the Director, Mr. W. M. Milliken.

These multiple lesions appeared to be multicentric rather than metastatic in origin. In none of these fish examined has there been any evidence of visceral metastasis.

Microscopic Examination

Histologically, the tumors were composed of long spindle cells arranged in broad interlacing bundles (Fig. 3). The surface epithelium rested directly upon the neoplastic cells and might send long pegs into the tumor, particularly when the growth was a small one (Fig. 4). Areas of myxomatous degeneration were found in which the cells appeared bipolar. In a large, slowly growing tumor on the caudal fin, one portion of the lesion showed palisading of the nuclei (Fig. 5), but the Bodian stain failed to reveal any trace of nerve fibrils. Van Gieson's stain and Milligan's trichrome stain [13] gave the reaction characteristic of smooth muscle rather than of connective tissue in all of the tumors. Myofibrils could be demonstrated in at least some of the cells in every neoplasm.

In the large, rapidly growing tumors many of the cells had lost their elongate character and were broad polygonal elements with abundant polychromatic cytoplasm and large oval nuclei that contained 2 to 4 prominent nucleoli (Fig. 6). These cells at times reverted to the characteristic spindle shape when the growth rate was reduced. This was demonstrated by the response of one of the tumors to transplantation. When a tumor fragment was placed in the anterior chamber of the right eye as an autotransplant, it grew slowly for a period of 3 months. At the end of that time the animal died; sections of the eye showed that the transplant had assumed the pattern of less rapidly growing tumors.

Occasionally, the large polygonal cells contained acidophilic intracytoplasmic inclusions (Fig. 7). Whether these merely represented foci of degeneration, or were indicative of the presence of a virus has not been determined. Multinucleated tumor giant cells were seen infrequently, but in some sections of the rapidly growing neoplasms they were a prominent element in a few areas (Fig. 8).

Growth in Tissue Culture

Attempts at *in vitro* culture of the tumor cells were successful with each of three large, rapidly growing tumors. A fourth tumor of this kind showed consistent contamination with a chromogenic bacterium, probably *Pseudomonas fluorescens*. Another large but stationary neoplasm, described above as showing palisading of nuclei in histologic sections, failed to grow in tissue culture. An effort was made also to cultivate small, slowly growing tumors *in vitro*, but without success.

Methods

The tissues were cultured at room temperature (19° to 23°C.) using the hanging-drop method and the roller tube technic of Gey.[14] The medium was the same as that most often employed for mammalian tissues: chicken plasma and beef or chick embryo extract for clot, with blood serum from human umbilical cord and Tyrode's saline solution as the liquid phase. The fluid in the roller tubes was changed every second day. Serial transplantation was not effected, but single cultures were kept under observation for as long as 8 weeks. To guard against infection, the surface of the tumor was first cut away and the tissue for explantation then removed and washed in several changes of Tyrode's saline solution. Like several other investigators, I employed penicillin to the amount of 1500 units per cc. in the Tyrode's solution used in the liquid medium. After several days, during which growth had begun, penicillin often was omitted from some of the cultures, but no effect on the pattern or rate of growth was observed.

Grand, Gordon, and Cameron [1] found that the melanoblastoma of a hybrid tropical aquarium fish grew better *in vitro* when fish serum was used in place of human cord serum. A similar effect was not apparent with the goldfish tumors when the blood of carp, a species very closely related to the goldfish, was used instead of human serum. Evidence of the remarkable indifference of goldfish tissue to the source of the culture media is shown by the behavior of adult heart muscle in media derived from human and bovine sources. Not only did the explants show active outgrowth of fibroblasts, but 18 days after transplantation the muscle began to beat rhythmically at the rate of once every 15 seconds. This ceased 6 days later, but the growth of fibroblasts continued.

Observations

On the second day after transplantation of the neoplastic tissue to a hanging-drop or roller tube, macrophages had migrated some distance from the explant. These wandering cells were identified by their almost circular outline, and by the presence of many highly refractile granules in the cytoplasm. After this emigration of the macrophages, large flat cells were seen growing out from the periphery of the explant. These had very finely granular or clear cytoplasm and large oval nuclei which after fixation were pale staining, with 2 to 4 nucleoli (Fig. 9). The cells might fuse to form multinucleated giant cells which resembled those seen in sections of the tumor (Fig. 10). Mitotic figures occasionally were observed; rarely, they were tripolar.

The general pattern of growth was that of a sheet of cells spreading

out from the explant, and was best seen in roller tube cultures (Fig. 11). The large cells were the predominant element in all cultures, whether growing in a hanging-drop or roller tube, and undoubtedly represented the neoplastic component. They bore a striking resemblance to the large cells seen in histologic sections of the rapidly growing primary tumors (Fig. 6).

Although the cytoplasm in the living cells usually was quite structureless, myofibrils sometimes were seen in the fixed and stained preparations. Tension striae (Fig. 12) were prominent in the large flat cells of some hanging-drop cultures. In the latter, occasional cells contained acidophilic cytoplasmic inclusions (Fig. 13). The significance of these structures has not been evaluated and the possibility that they represent merely phagocytized protein cannot be overlooked. The resemblance which they bear to similar inclusions found in the tissue sections is striking (Fig. 7).

In several of the hanging-drop preparations there were many cells with hyperchromatic nuclei and condensed cytoplasm forming a fibrillated strand which lent the cell a very long spindle shape or even a bipolar character (Fig. 14). A cytoplasmic vacuole which deformed the nucleus was of frequent occurrence. When examined stereoscopically, the relationship between these cells and the large flat ones became clear. Long spindle cells were the only type found in the depth of the culture, where they were surrounded by coagulum. When one of these cells came in contact with the overlying coverglass, the cytoplasm spread out in a broad thin film; the nucleus, too, became flattened and the chromatin appeared less dense. The cells had then become indistinguishable from the large polygonal cells described above.

As early as 90 hours after explantation, sprouts of endothelium appeared in the proliferating zone of cells (Fig. 10). Behind the growing tip, a lumen separated the adjacent cells, producing a rather well formed capillary. These could always be traced back to the explant and were outgrowths from capillaries present in the tumor at the time of transfer. After several weeks these channels might form an extensive branching network. The caliber of the lumen was fairly uniform and usually smaller than, although occasionally equal to, that observed in the cutaneous capillaries of the living fish.

As previously noted, the cultures were kept at room temperature which varied between 19° and 23° C. Twelve actively growing roller tube cultures were placed in an incubator at 37° C. Growth continued for 3 days but thereafter ceased and regressive changes set in. During the next 17 days a control group kept at room temperature continued

to grow, while those in the incubator reached advanced stages of degeneration. When returned to room temperature these cultures failed to initiate new growth.

Discussion

Tumors in fishes are not very uncommon, but in view of the widespread use of goldfish as pets and for ornamental purposes, it is rather surprising that only 13 reports of neoplasms in these fish are found in the literature.[2] In 10 the lesions were identified as fibromas or fibrosarcomas of the corium or subcutaneous connective tissue. Although there is no record of leiomyomas in goldfish, it is possible that some of the tumors identified as of connective tissue origin were in fact derivatives of smooth muscle.

The question of the benign or malignant nature of the leiomyomas is not easily answered. Certainly the small, slowly growing, usually multiple neoplasms must be classified as benign. The large, rapidly growing tumors display many characteristics of malignant lesions; *e.g.*, many mitotic figures, large nuclei with prominent nucleoli, and invasion of surrounding tissues. Metastases were not observed, but these are uncommon in fishes; the entire literature contains only 14 instances in which a primary tumor had metastasized,[2] none being reported in goldfish. The reason for this absence of metastasis is obscure and the explanation commonly given, *viz.*, that the tumor-bearing and therefore handicapped fish are destroyed before the lesion has had time to metastasize, is scarcely pertinent in this instance, since the animals were carefully tended in aquaria while the tumors increased rapidly in size.

Although the neoplastic smooth muscle cells were grown readily *in vitro*, at no time did they show evidence of contraction. That the media used permitted development of normal structure and function is indicated by the resumption of rhythmic contraction by a bit of adult goldfish heart 18 days after explantation. The absence of contraction may be attributed in part to the fact that most of the smooth muscle cells were spread out flat against the coverglass. In her study of smooth muscle cells grown from chick amnion, M. Lewis [3] observed that the flattened cells usually failed to contract; whereas, she saw occasional rhythmic contractions in the elongated and band-like cells near the explant.

Myofibrils were not observed in the living tumor cells and could be identified only with difficulty in the fixed and stained preparations. M. Lewis [3] also was unable to recognize myofibrils in living smooth muscle cells cultured from chick amnion, although they were readily visible after fixation. Similar findings are recorded by W. Lewis [4] in

his study of embryonic chick heart explants, which frequently beat rhythmically in the absence of both cross and longitudinal striations. Champy,[5] employing urinary bladder and arterioles of the adult rabbit, found that the smooth muscle cells present in his cultures did not contract and were without fibrillae even after fixation; therefore they were indistinguishable from fibroblasts. Bloom [6] has suggested that the use of adult tissues by Champy and of embryonic tissues by the Lewises accounts for the discrepancies in their results. The neoplastic smooth muscle cells of fish bear a striking resemblance to those of the amnion of the chick in size and shape, and in the presence of occasional fibrillae after fixation. Their lack of functional activity more closely resembles that of cells of the adult rabbit which likewise failed to contract.

The tension striae seen in some of the cells (Fig. 12) are similar to those described by the Lewises,[7] not only in heart and smooth muscle cells, but also in endothelium and mesothelium. The striae appear to be folds in the surface of the cell produced by tension and reversible when this is relaxed. The effect upon the cell of surface forces acting at the interface of glass and cytoplasm is indicated not only by the presence of tension striae but by the large flat shape which the cell assumes. When growing in fibrin clot these same cells appear as elongated spindles (Fig. 14).

The source of the neoplastic smooth muscle cells has not been identified with certainty, but is probably the walls of blood vessels. This site has been accepted by Stout [8] for the majority of solitary cutaneous leiomyomas in man. In the multiple cutaneous leiomyomas reviewed by Ormsby,[9] the tumors arose chiefly in the arrectores pilorum. Stout pointed out that the human cutaneous leiomyomas may develop in the walls of larger veins but not of arteries. He believed that the tumor vessels are neither normal veins nor normal arteries, and that muscle bundles of the vessels merge with the neoplastic muscle so frequently that the origin of the latter from the former seems evident.

The corium of the goldfish is very vascular, with surprisingly large arterioles present just below the epidermis. The muscle in the walls of these vessels closely resembles that seen in the tumors. Search for arteriovenous shunts in sections of the corium as well as in the caudal fin of living fish has been fruitless. In 1888 Mayer [10] gave a detailed account of peculiar doughnut-shaped, isolated masses of smooth muscle that surround arterioles in the subcutaneous tissue and viscera of certain selachians, especially the skate *Raja clavata*. No similar structures were seen in the corium of goldfish, nor could a report of their occurrence in teleost fishes be found in the literature.

All of the tumor-bearing goldfish described in this report have been obtained from the same pond. Many more fish were observed with tumors than were collected; an effort is being made to preserve this stock for further observations on its life history and ecology. Despite careful inquiries among local aquarists and the Ohio State Department of Conservation, no other pools with tumor-bearing goldfish could be located in this vicinity. The rather high incidence of this unusual tumor in one species of fish in a single pond points to an extrinsic agent acting perhaps on a highly susceptible inbred strain. The occasional cytoplasmic inclusions observed in the tumor cells lend support to this possibility.

An infectious etiology for certain tumors of goldfish has been suggested by two groups of investigators. In a tank containing 20 goldfish, Roffo [11] found 7 with neoplasms that he identified as fibrosarcomas. He found proof of the infectious character of the tumor in the fact that neoplasms did not develop among goldfish placed in the tank after it had been sterilized and the diseased animals removed. Montpellier and Dieuzeide,[12] in their report of fibrosarcomas in each of 5 goldfish from the same pool, also raised the question of an infectious agent.

Summary

Approximately 3 to 4 per cent of the goldfish in a large pond bore single or multiple leiomyomas.

The tumors probably arose in the walls of blood vessels and some reached a diameter of 5 cm. Such large, rapidly growing neoplasms were locally invasive and histologically malignant. No metastases have been found.

The general pattern of growth in tissue culture was that of a sheet of cells spreading from the explant. Large flat cells fused to form giant cells similar to those seen in sections.

Acidophilic inclusions were present in the cytoplasm of occasional tumor cells; these were seen in histologic sections of the neoplasms as well as in cells growing *in vitro*.

It is suggested that these tumors may be due to an infectious agent acting on a highly susceptible inbred strain of fish.

Technical assistance in the preparation of the tissue cultures and photographs by Miss Roberta Walker and Mrs. Ralph Lewis is gratefully acknowledged.

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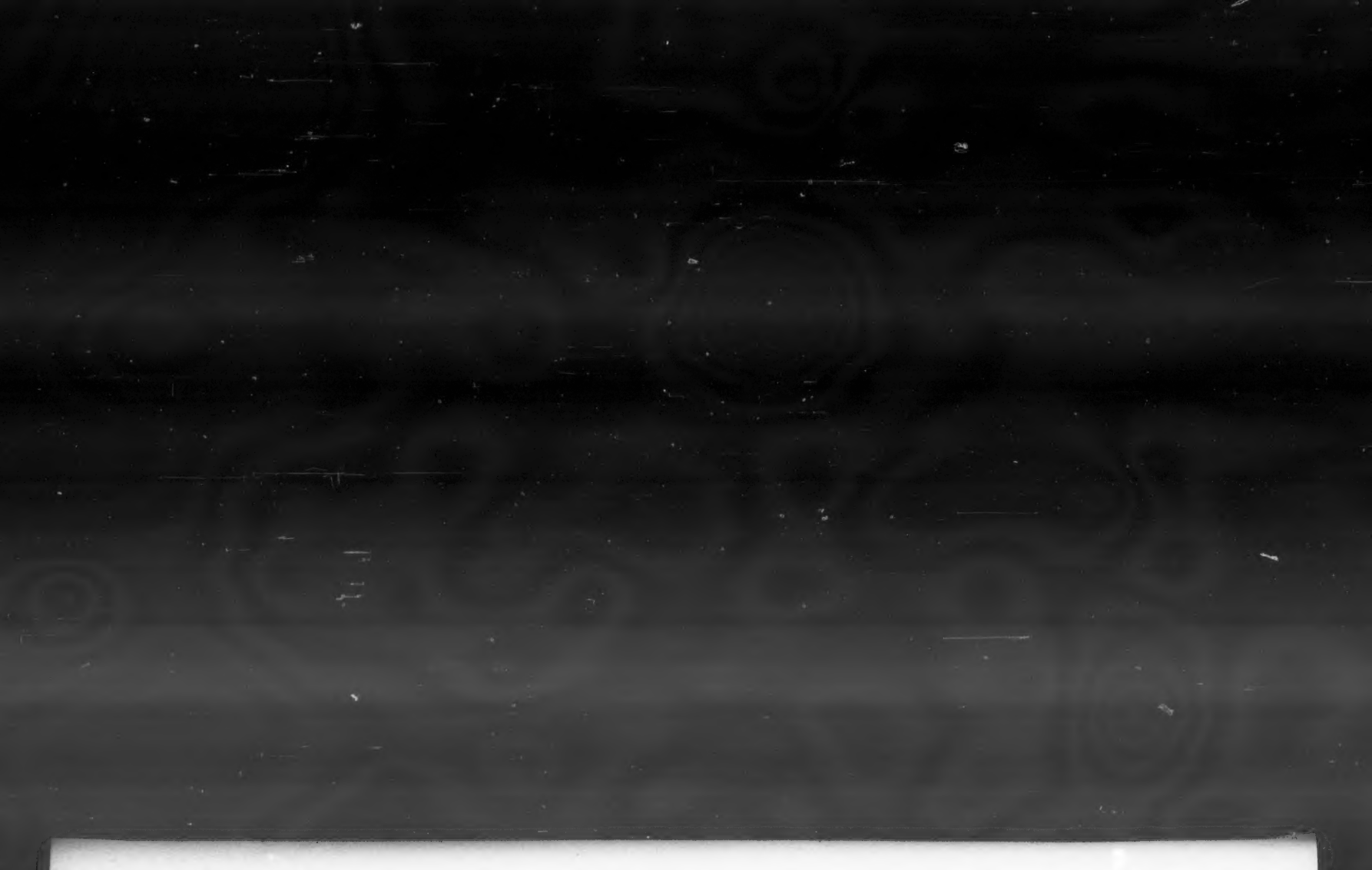
DESCRIPTION OF PLATES

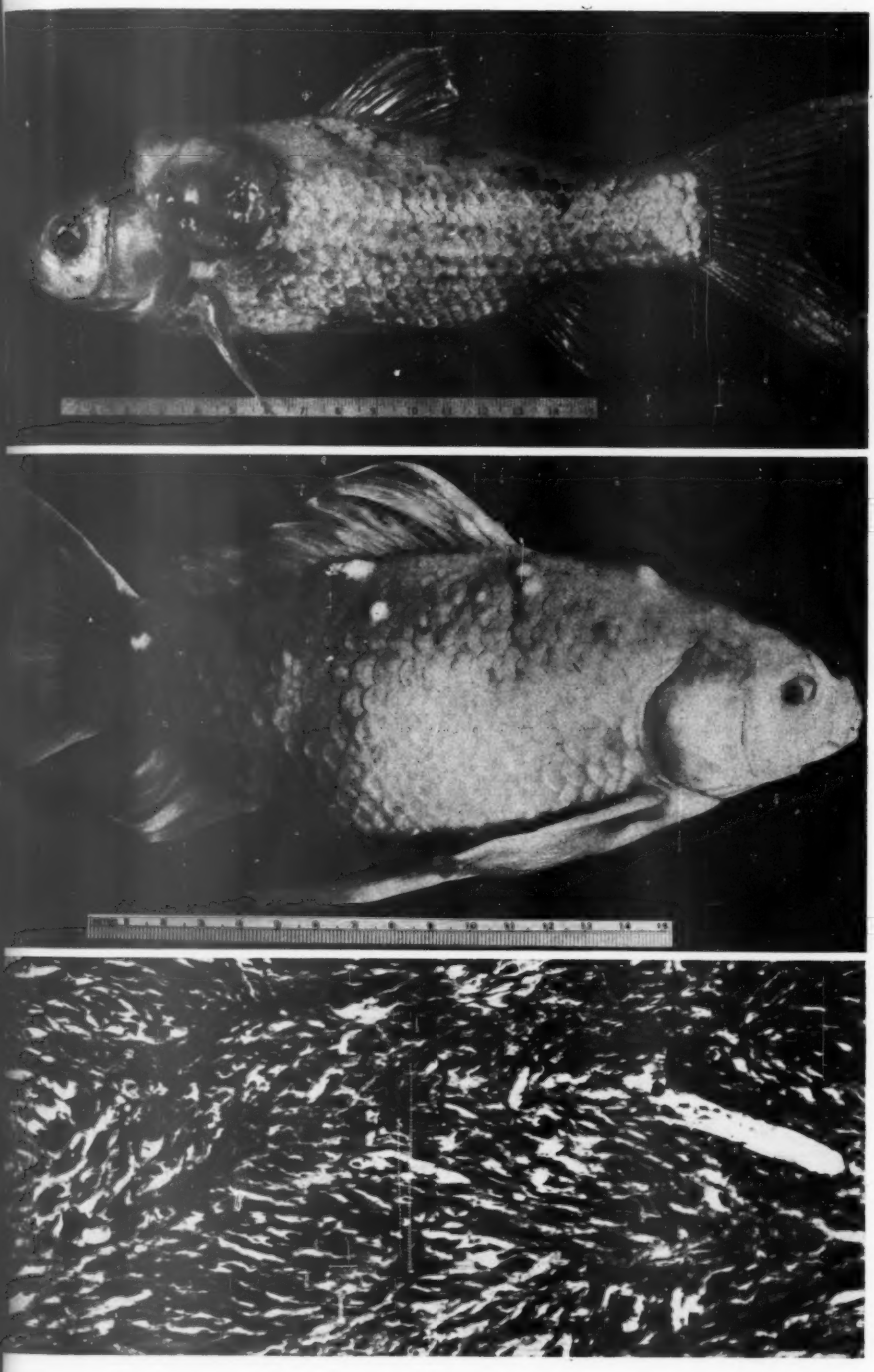
PLATE 38

FIG. 1. A rapidly growing tumor on the trunk, directly behind the operculum. The fish had been under observation for 57 days, during which time the tumor doubled in size.

FIG. 2. Multiple leiomyomas are present on the trunk, caudal and dorsal fins, and operculum.

FIG. 3. Section illustrating the characteristic pattern of interlacing bundles assumed by the spindle-shaped tumor cells. Milligan's trichrome stain. × 275.





Schlumberger Leiomyoma of Goldfish

PLATE 39

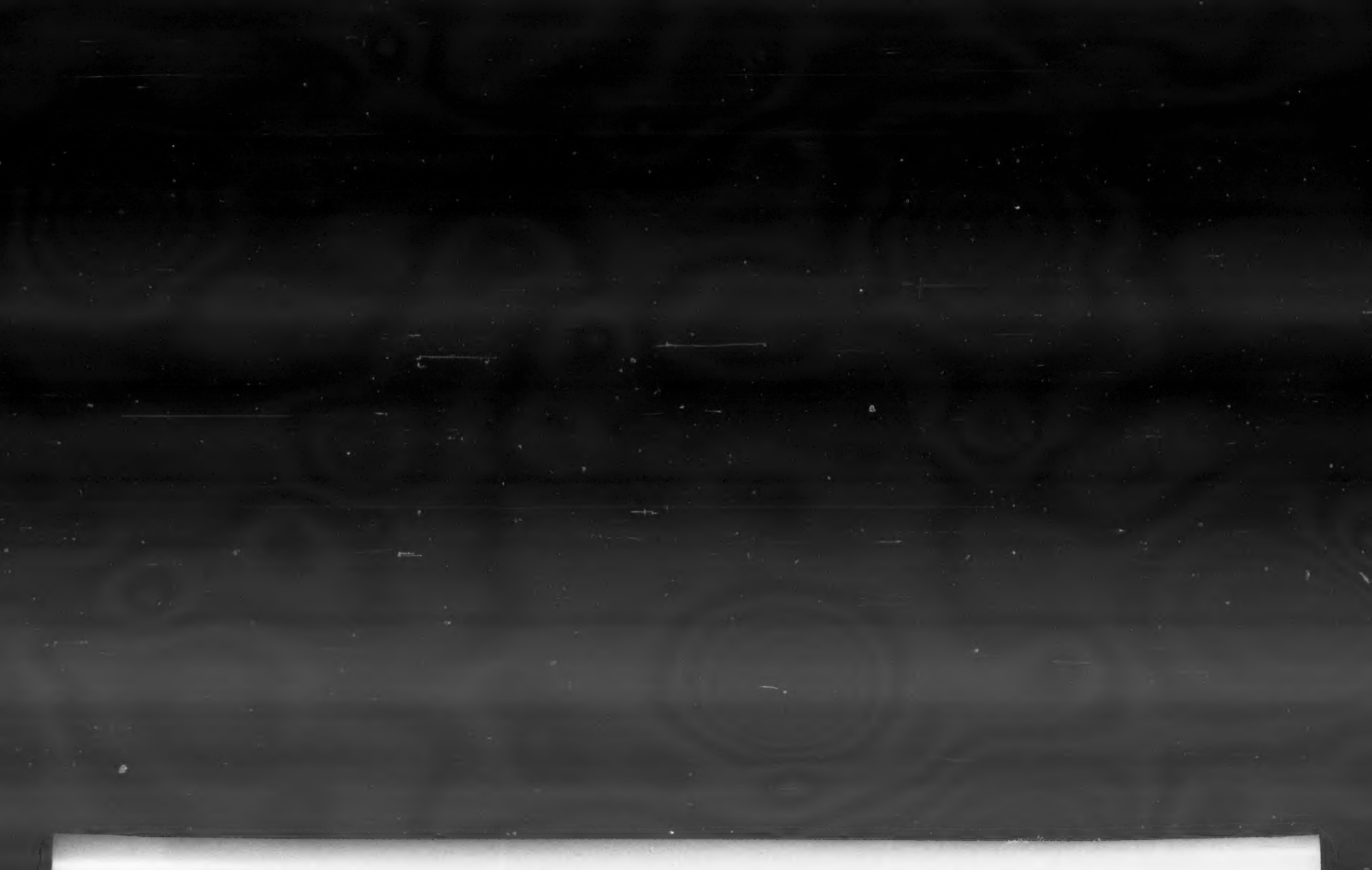
FIG. 4. Section through a small cutaneous nodule showing two epithelial pegs extending deeply into the tumor. The neoplastic smooth muscle cells are in intimate contact with the epidermis. Myxomatous degeneration, lending many of the cells a bipolar character, is prominent. Hematoxylin and eosin stain. × 100.

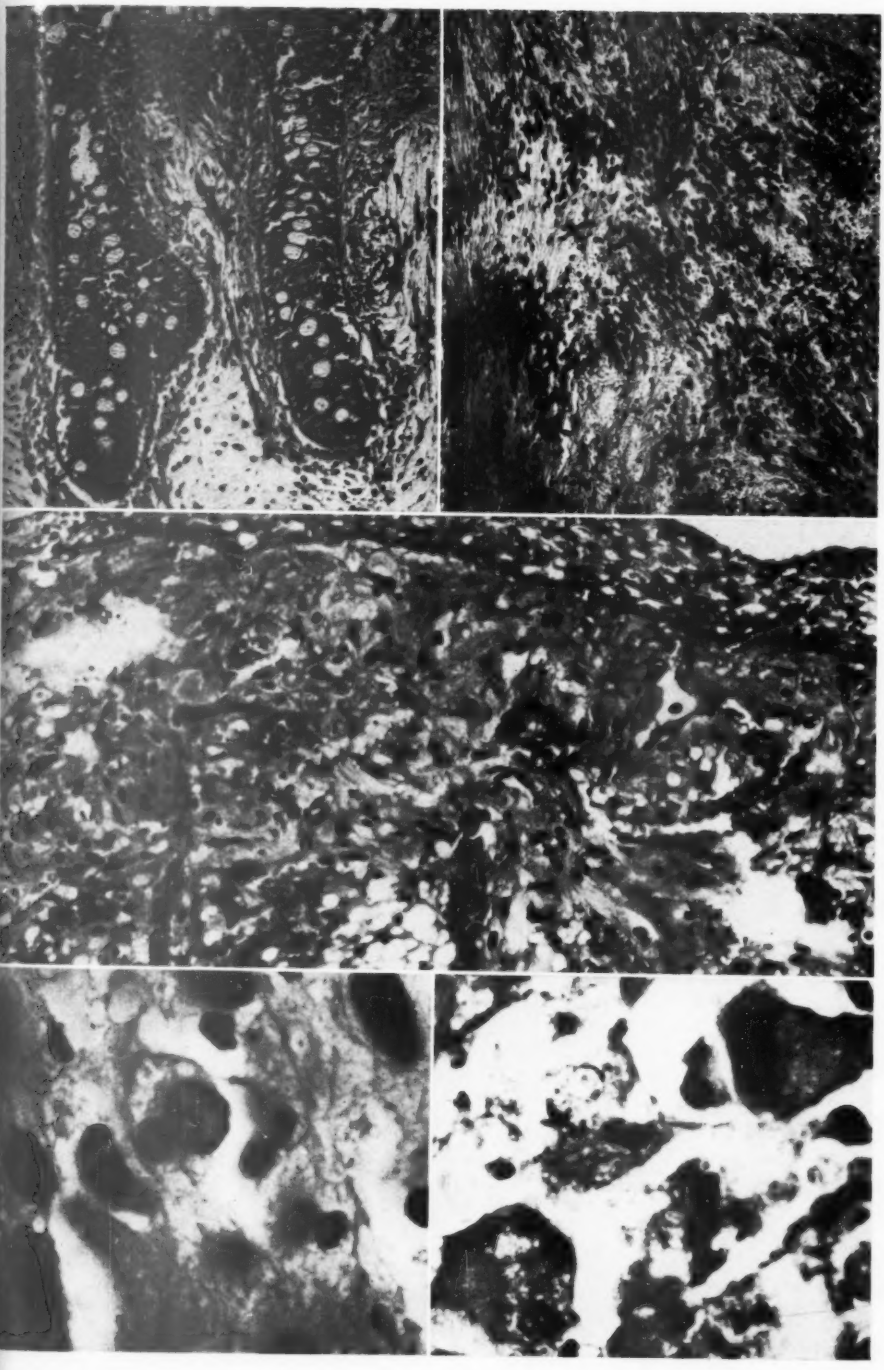
FIG. 5. Palisading of the nuclei is present in one portion of a slowly growing tumor, 2.5 by 2 by 2 cm., on the caudal fin. The Bodian stain failed to reveal any trace of nerve fibrils. Hematoxylin and eosin stain. × 150.

FIG. 6. Section of a rapidly growing tumor, 3.5 by 2.5 by 2 cm., on the dorsum of the head. The cells possess an abundant polychromatic cytoplasm, are in direct contact with the epidermis, and contain large nuclei with finely dispersed chromatin. Hematoxylin and eosin stain. × 275.

FIG. 7. Acidophilic cytoplasmic inclusion body in a cell from the tumor shown in Figure 6. Seller's basic fuchsin stain.[15] × 950.

FIG. 8. Multinucleated tumor giant cells. Hematoxylin and eosin stain. × 700.





Schlumberger Leiomyoma of Goldfish

PLATE 40

FIG. 9. Characteristic appearance of tumor cells when in contact with the coverglass. There is an abundant granular cytoplasm; in the living cell such coarse granularity is not present. The chromatin is finely dispersed within the nuclei. In the upper right corner of the photomicrograph a cell is undergoing mitosis. Hanging-drop culture, 162 hours after transplantation. Hematoxylin and eosin stain. × 700.

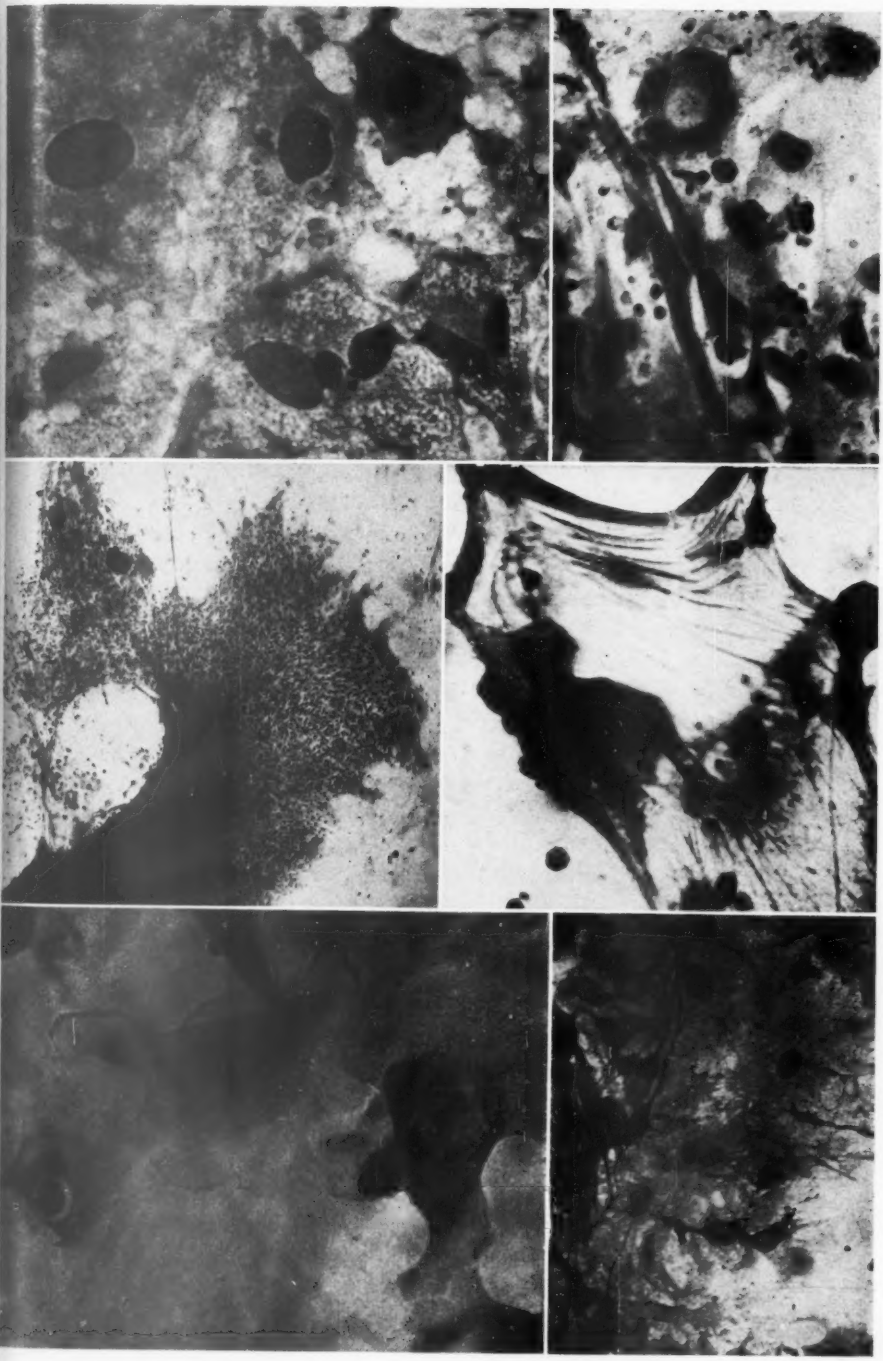
FIG. 10. A strand of endothelial cells passes diagonally across the field. Touching it at one point is a circlet of nuclei which may represent a tumor giant cell. Multiple, linearly arranged nucleoli are clearly visible in the nuclei. Hanging-drop culture, 90 hours after transplantation. Hematoxylin and eosin stain. × 500.

FIG. 11. Sheet-like outgrowth of tumor cells in a roller tube culture, 8 days after transplantation. Hematoxylin and eosin stain. × 80.

FIG. 12. Tension striae in the cytoplasm of neoplastic smooth muscle cells, 90 hours after transplantation. Hanging-drop culture. Hematoxylin and eosin stain. × 500.

FIG. 13. Acidophilic cytoplasmic inclusions in tumor cells grown for 90 hours in a hanging-drop culture. Hematoxylin and eosin stain. × 700.

FIG. 14. The cells that are no longer in contact with the coverglass have acquired a very long spindle shape somewhat suggestive of bipolar nerve cells. However, transition stages between these and the large flat polygonal cells are numerous. Hanging-drop culture, 162 hours after transplantation. Hematoxylin and eosin stain. × 350.



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Schlumberger Leiomyoma of Goldfish

THE EFFECTS OF DEPRIVATION OF WATER ON THE ADRENAL GLANDS OF RATS *

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In recent years many papers have appeared dealing with the reaction of the adrenal gland to various forms of physiologic stress. So far as I can ascertain, there have been no published reports on the effects of acute dehydration. It was with this in mind that the following study was undertaken.

MATERIALS AND METHODS

Sixteen female albino rats of the Wistar strain were used. These rats weighed about 300 gm. each, with none varying more than 10 gm. Five were killed with chloroform and designated as normal. The remainder were kept in screen-bottomed cages and allowed access to purina laboratory chow at all times. No water was allowed. On the 8th day 4 animals were killed with chloroform. One rat died on the 9th day, 4 on the 10th day, and one each on the 11th and 12th days.

When each animal died or was killed the adrenal glands were removed immediately and immersed in 10 per cent formalin. After fixation for 24 hours they were rinsed in tap water, and the surrounding fat was carefully dissected away. One gland from each animal was used for histologic study and the other for chemical determinations. The gland which was chosen for histologic study was embedded, cut by the freezing method, and mounted by the method of Zwemer.[1] For demonstration of total fats, the sections were stained with sudan III by the method of Romeis.[2] For demonstration of cholesterol content the Schultz reaction was used.[3] The method used with the other gland for the quantitative chemical determination of total fats and cholesterol has been described previously.[4]

RESULTS

The results obtained from the quantitative analysis are tabulated and illustrated graphically (Text-Fig. 1). There was a marked decrease in total fats and cholesterols as dehydration proceeded. However, the weights of the glands and the water content were remarkably constant (Table I).

The histologic picture as revealed by the sudan III and the Schultz methods closely followed the quantitative findings. The distribution of total lipids in the normal gland is shown in Figure 1. Here may be seen a well outlined zona glomerulosa staining heavily with sudan; just inside

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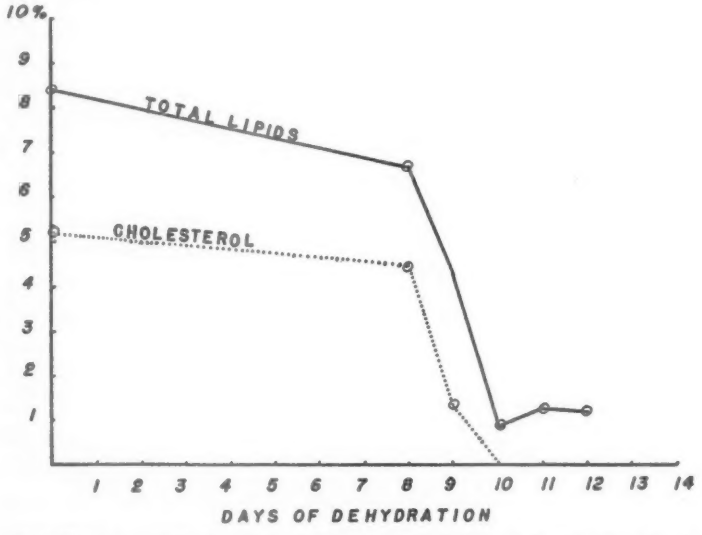
there is a narrow, lightly staining zone. The zona fasciculata is also heavily stained, but the stain decreases in intensity as the zona reticularis is approached. The zona reticularis stains somewhat lighter than the zona fasciculata and it grades off until it reaches the medulla, where it ends abruptly. The Schultz reaction for cholesterol closely paralleled that of the sudan stain for total fats.

In the rats which had been dehydrated for 8 days there was found essentially the same condition in the adrenals as in the normal animals, except that the intensity of the sudan and Schultz stains was slightly less throughout.



Text-Figure 1. Depletion of total lipids and of cholesterol in the adrenals of rats deprived of water.

The rat which died on the 9th day showed marked changes (Fig. 2). In a section of the adrenal from this animal, stained with sudan for total fats, the zona glomerulosa is seen to be intact and well outlined although staining somewhat less densely than in the normal animals. The total lipids of the zona fasciculata and outer reticularis have become markedly depleted, while the inner reticularis is somewhat less disturbed. The Schultz reaction for the cholesterol fraction showed that the cholesterols had become depleted in the same manner and distribution as the total lipids.

The adrenals of the rats which died on the 10th, 11th, and 12th days showed the greatest change. Lipids were almost entirely absent in all

TABLE I

The Weight and Lipid Content of the Adrenal Glands of Dehydrated Rats

Treatment	Rat no.	Wet weight, adrenal	Dry weight, adrenal	Water	Ether-extracted weight	Fat	Cholesterol weight	Cholesterol
		mg.	*mg.*	*per cent*	*mg.*	*per cent*	*mg.*	*per cent*
Normal	1	18.1	6.9	61	5.4	8.5	0.780	4.3
Normal	2	16.0	6.3	61	4.8	9.3	0.885	5.5
Normal	3	12.1	4.8	60	3.7	9.0	0.700	5.8
Normal	4	15.1	6.0	60	4.6	9.2	0.855	5.6
Normal	5	14.7	5.2	64	4.3	6.1	0.735	5.0
Average						8.4		5.2
Dehydrated								
8 days	6	11.8	5.0	57	4.2	6.8	0.650	5.5
8 days	7	14.3	5.8	59	4.7	7.6	0.625	4.3
8 days	8	14.0	6.1	56	4.8	8.4	0.625	4.4
8 days	9	14.9	5.8	61	6.4	4.0	0.600	4.0
Average						6.7		4.5
Died of dehydration								
9th day	10	18.5	7.4	60	6.6	4.3	0.250	1.3
10th day	11	21.2	7.9	62	7.8	0.5	Too low to read colorimeter	
10th day	12	17.6	6.4	63	6.2	1.0	Too low to read colorimeter	
10th day	13	20.5	8.1	60	7.9	0.9	Too low to read colorimeter	
10th day	14	20.1	8.2	54	7.9	1.4	Too low to read colorimeter	
11th day	15	22.1	8.7	60	8.4	1.3	Too low to read colorimeter	
12th day	16	15.4	5.8	62	5.6	1.2	Too low to read colorimeter	
Average						1.5*		

* 1.05 per cent for 6 animals surviving 10 days or more.

of the zones, only a few lipid droplets remaining in the zona glomerulosa. The Schultz reaction revealed that the cholesterols had entirely disappeared. These glands are not illustrated but the chemical findings are indicated in Table I.

Discussion

Most investigators who have subjected animals to stresses of the general type considered here have found marked depletion of lipids of the adrenals, especially in the inner zones. Dosne and Dalton,[5] studying the effects of cold, fasting, and the injection of formaldehyde, obtained depletion of lipids, as did Oleson and Bloor [6] who studied fasting. Andersen,[7] and Knouff, Brown, and Schneider [8] obtained lipid depletion following or during increased muscular activity. Levin,[9] Langley and Clarke,[10] Giragossintz and Sundstroem,[11] Darrow and Sarason,[12] Dalton, Mitchell, Jones, and Peters,[13] Tepperman, Tepperman, Patton, and Nims,[14] and Nichols [4] obtained depletion of lipids in the inner zones during exposure to anoxia. Ludewig and Chanutin [15] found that prolonged chloroform anesthesia causes depletion of lipids. Vogt[16] found the same after injection of insulin. These reactions are considered by Selye [17] to be part of the "alarm reaction."

I am unable to give a definite explanation for the changes observed. However, there are certain reported observations which may be related. Marriott [18] has demonstrated that in the early stages of anhydremia there is an increase of blood proteins and in the advanced stages a decrease in blood proteins together with a high nonprotein nitrogen. Dougherty and White,[19] in a series of publications, have shown that the 11-oxycorticosteroids secreted by the zona fasciculata and reticularis cause a dissolution of lymphatic tissue and an increase of blood protein, especially in the gamma globulin fraction. Since 100 gm. of protein can give rise to 45 gm. of endogenous water, the changes in the adrenal described in this paper may be part of an attempt by the organism to provide itself with more water without the use of exogenous food. The fascicular and reticular zones, first affected in the experiments reported here, are known to be under the control of the pituitary gland (the zona glomerulosa is not). This has been demonstrated by Smith,[20] Crooke and Gilmour,[21] Sarason,[22] and many others, who found atrophy of these two inner zones after hypophysectomy. This atrophy can be prevented by administration of adrenotrophic hormone (Meyer, Mellish, and Kupperman [23]).

Conclusion

Marked depletion of total lipids and especially of cholesterols was found in the adrenal cortices of rats subjected to prolonged dehydration. This depletion was especially pronounced in the zona fasciculata and

zona reticularis. The zona glomerulosa was the last to become depleted of lipids. The histologic observations were found to be closely paralleled by quantitative chemical findings.

ADDENDUM

It has recently been pointed out by Schmidt-Nielsen, Schmidt-Nielsen, and Schneiderman [24] that certain desert mammals, especially the kangaroo rat (*Dipodomys merriami*), do not require water; in fact these animals can live indefinitely on desiccated grain. Furthermore, these animals can excrete urine with a 0.908 normal chloride concentration. This is one and one-half times the normal maximum for the laboratory rat and three times that of man. Experiments are being carried out on this animal in this laboratory.

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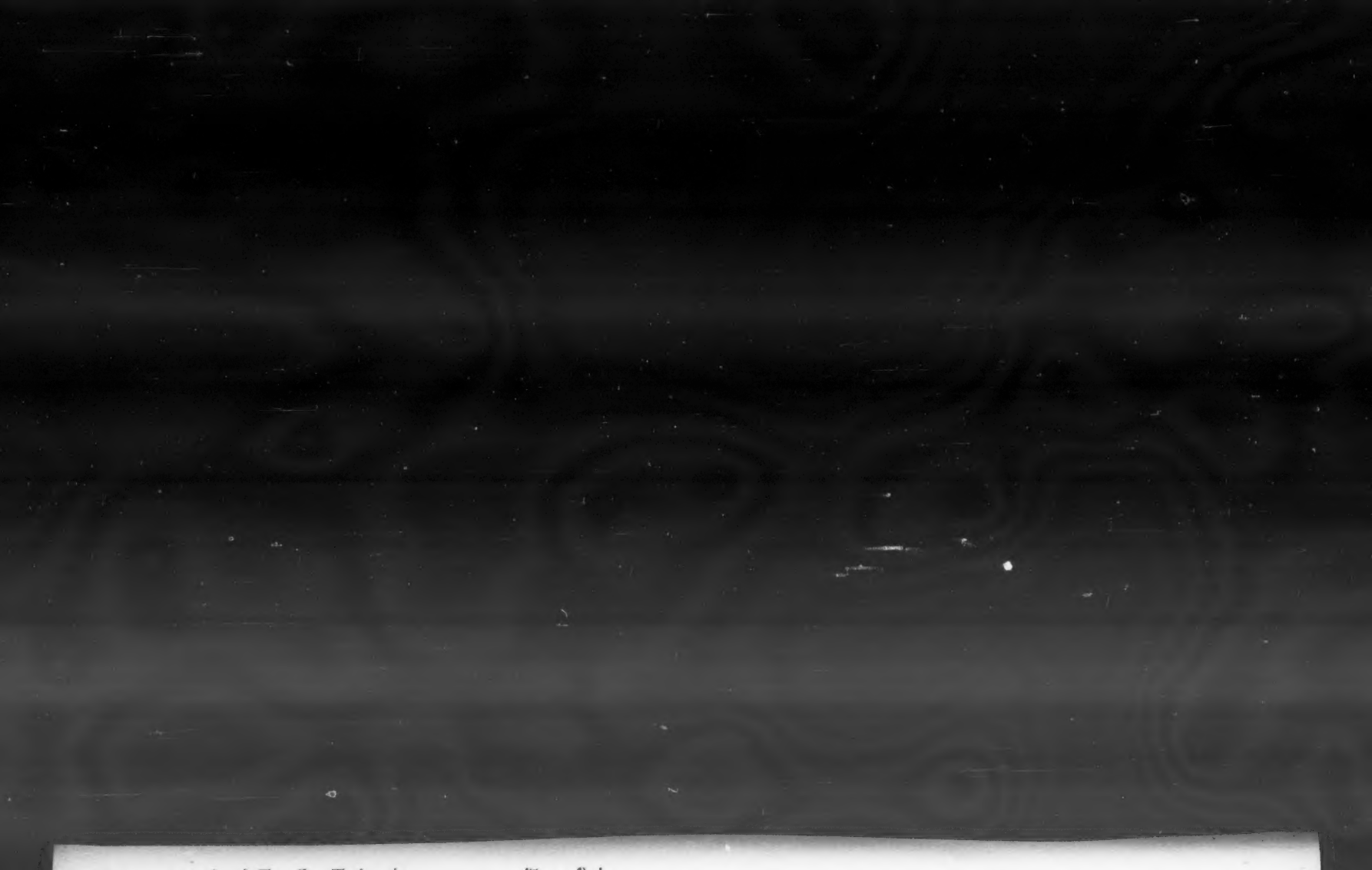
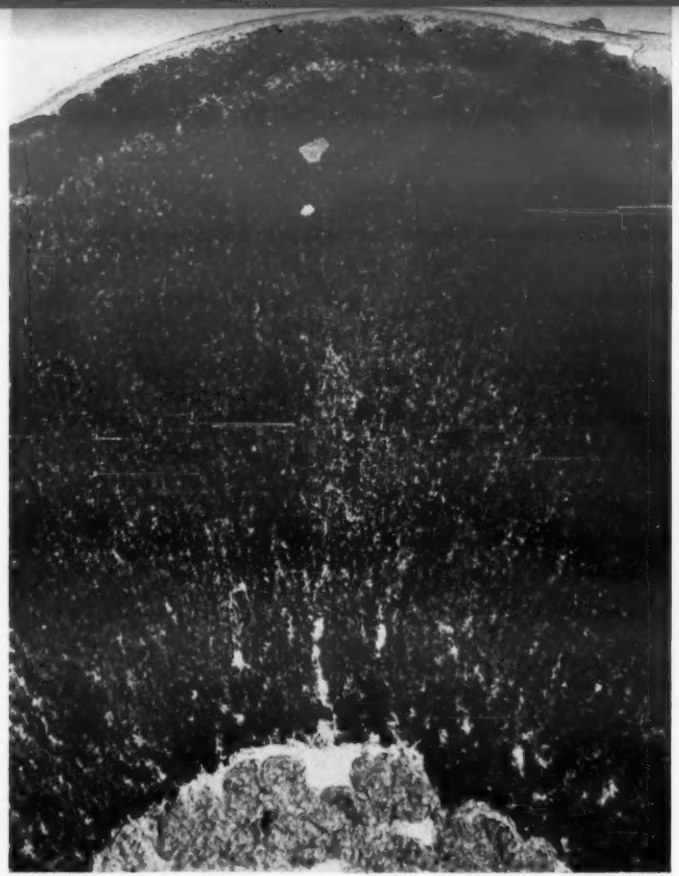
DESCRIPTION OF PLATE

PLATE 41

FIG. 1. Adrenal gland from a normal animal, showing the normal distribution of lipids. Sudan III stain. × 135.

FIG. 2. Adrenal gland from dehydrated animal 10, showing depletion of lipids in the inner zones, especially the zona fasciculata and outer portion of the zona reticularis. Sudan III stain. × 135.





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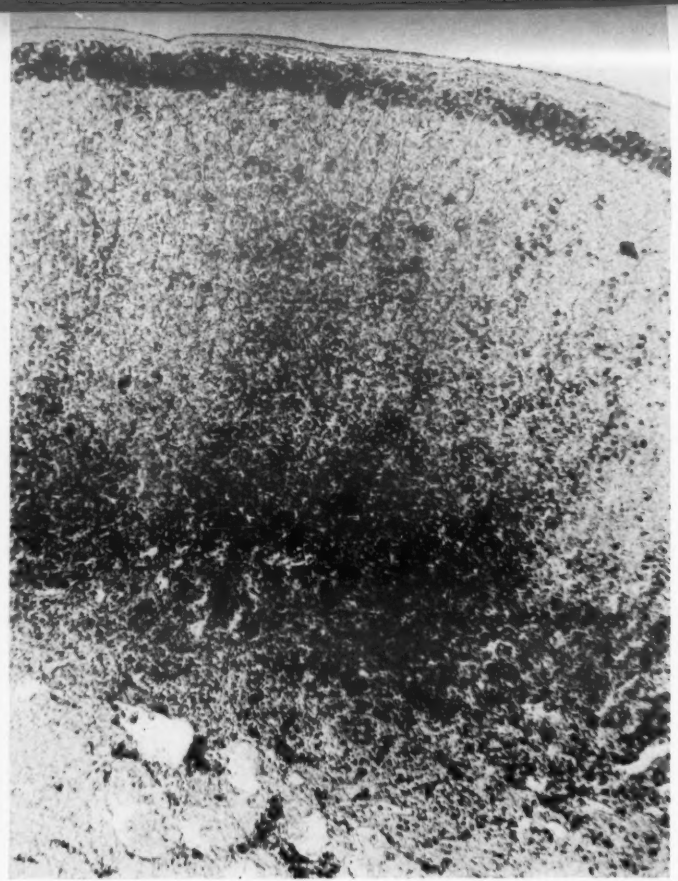

2

PLASMA CELL HYPERPLASIA AND HYPERGLOBULINEMIA IN TRICHINOSIS

The Duration of Larviposition *

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Because of certain clinical manifestations, a patient at the National Naval Medical Center was thought to have multiple myeloma, and it was not until an autopsy was performed that the true nature of the disease process—trichinosis—was ascertained. It is believed that this is the first recorded instance in which plasma cell hyperplasia and hyperglobulinemia have been observed in a patient infected with *Trichinella spiralis*. The question naturally arises as to whether the plasma cells were a manifestation of an inflammatory response to the nematode, or whether an incipient form of multiple myeloma was developing simultaneously. For reasons later to be discussed, a neoplastic process, in all probability, is untenable.

Of perhaps more significance, especially from a clinical viewpoint, is the maximal duration of the intestinal phase in the human host. It is obvious that so long as gravid females are present in the intestinal tract, larvae will continue to migrate through the tissues, thus precluding recovery. Adult worms in the intestine have been reported in only a few cases,[1-7] and in some, microscopic confirmation has been lacking. Six weeks is the time factor generally stated for the duration of larviposiing by adult female trichinae, but this figure has been determined principally from studies in animals, particularly guinea-pigs.[8]

Conclusions drawn from animal experiments generally are not applicable to human trichinosis since there are definite species differences which modify the duration of the disease. In addition, the number of cysts ingested, the number of previous exposures, and the immunity acquired by these exposures considerably alter the host-parasite relationship, especially with respect to the viability of trichinae. Horlick and Becknell [6] reported a case of trichinosis, the duration of the infection of which was 30 days; adult worms in the intestine were confirmed by microscopic examination. Recently, Stryker [9] presented a case with, up to that time, the longest period of persistence of adult trichinae in the human intestine. The adult trichinae, including gravid females, were demonstrated in the intestine 54 days after ingestion of infected pork. This author emphasized, and the case to be presented reaffirms, the im-

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portance of the continued release of larvae over even possibly greater periods of time in the therapeutic management of trichinosis. In the case which follows, adult trichinae, including gravid females, were observed microscopically in the mucosa of the duodenum and ileum 115 days after the onset of symptoms.

Report of Case

N. P., a 61-year-old veteran of World War I, was admitted to the orthopedic service of the U. S. Naval Hospital on March 4, 1946, with the chief complaint of "pain in the back." He had been in good health until 4 weeks previously when he developed a moderately severe nonproductive cough which lasted 5 days. He denied gastro-intestinal complaints. About 3 weeks before admission, he experienced sharp, persistent pain and spasm in the muscles of the back, first on the right side and then on the left. The pain, occasionally relieved by lying flat in bed, was aggravated by palpation and active motion. Pain or tightness in other muscle groups was denied. At no time had he noted skin rashes, hemorrhages, or periorbital edema. The patient lived alone. A history of ingestion of poorly cooked or raw pork was not obtained.

On admission, physical examination revealed a rather poorly nourished white male, weighing 140 pounds. He was well oriented and complained bitterly of back pain. The skin was flabby but no rashes, eruptions, discolorations, or scars were present. There was no edema, pallor, cyanosis, or dyspnea. The extra-ocular movements were normally performed, and elicited no pain. The heart was normal with respect to size, rate, and rhythm. No murmurs were heard. The blood pressure measured 130/70 mm. of Hg. The lungs were clear to percussion and auscultation. The general gait and posture supported the significant findings of an intermittent painful spasm of low back muscles, and local tenderness. True sciatic radiation was absent. Roentgenograms of the chest, spine, pelvic girdle, skull, and long bones revealed only moderate diffuse osteoporosis compatible with the patient's age and state of nutrition.

The admission laboratory examinations gave the following results: Kahn test, negative; urinalysis, unremarkable except for 10 mg. per cent of albumin; sedimentation rate, 30 mm. in 1 hour with a maximum fall of 17 mm. in 5 minutes; erythrocyte count, 3,100,000 per cmm.; hemoglobin, 9.5 gm. (66 per cent); leukocyte count, 6,000 per cmm. The differential count revealed 52 per cent neutrophilic polymorphonuclear leukocytes, 21 per cent lymphocytes, 12 per cent monocytes, and 15 per cent eosinophils. The erythrocytes showed marked hypochromia and slight anisocytosis. Because of the rather striking rouleaux formation, repeated tests for Bence-Jones protein were performed, but none was found.

On March 13, 1946, application of a spinal brace made the patient more comfortable. He had been receiving physiotherapy, and by March 28th his condition was considerably improved. This improvement continued until April 15th, when the patient complained of sharp pain in both hips with radiation down the posterior aspect of both thighs. The pain persisted, and on April 22nd he received a bilateral hip cuff injection of 2 per cent procaine, but obtained no relief. On April 25th, the total serum proteins were 12.8 gm. per cent, with 5.4 gm. per cent of albumin, and 7.6 gm. per cent of globulin.

The patient continued to suffer back pain, and on May 6th, the 63rd hospital day, he was transferred to the medical service for further investigation of his anemia and hyperglobulinemia. At that time his temperature, pulse, and respirations were normal and had been so since admission. The sedimentation rate was markedly elevated, and the urine continued to show a small amount of albumin. Occult blood was observed repeatedly in the stools. Hyperglobulinemia and hypochromic anemia

persisted, but despite repeated differential blood counts, the 15 per cent eosinophilia present on admission was not observed again. The values of all other laboratory tests, except those mentioned, were within normal limits prior to the terminal episode.

All diagnostic studies were interrupted during the patient's third day on the medical service, May 8th, by the sudden development of a severe pulmonary infection. Roentgenograms of the chest confirmed the clinical diagnosis of consolidation of the right lung with pleural effusion. The patient was treated with penicillin, oxygen, whole blood transfusions, and intravenous fluids. Despite this, his course was progressively and rapidly downhill. Two days before death, he became lethargic and stuporous. Coma developed, and he expired on May 30, 1946, the 87th hospital day.

Gross Autopsy Findings

Necropsy was performed 7 hours after death. The body was that of a well developed but emaciated 61-year-old white male. The subcutaneous fat was brilliant orange, and in some areas serous atrophy was pronounced. The musculature throughout the body showed marked loss of turgor. It was muddy brown, and some muscle groups, particularly the pectoralis, had the consistency of thick putty. Myriads of oval, friable, yellowish white, encysted trichinae, which measured approximately 0.5 mm. in length, were distributed throughout all muscle groups examined, including those of the head, neck, extremities, and the extra-ocular, pectoralis, trapezius, intercostals, diaphragm, and psoas muscles.

The heart weighed 300 gm. Serous atrophy of the epicardial fat was marked. The cardiac musculature was of normal thickness, but it was muddy brown and decidedly flabby. No encysted trichinae were observed, and there was no evidence of fibrosis or infarction. The coronary and great vessels showed moderate atheromatous changes.

The left lung weighed 600 gm.; the right, 680 gm. The lower lobe of the left lung and the middle and lower lobes of the right lung were voluminous, and on section, yellowish gray, elevated, granular areas of consolidation with numerous abscesses and areas of cavitation were found. The bronchi and bronchioles were congested and contained much mucopurulent material. The tracheobronchial lymph nodes were soft and enlarged.

The mucosa of the entire small bowel was markedly congested and edematous. An occasional punctate area of hemorrhage was found, but no areas of erosion or ulceration could be demonstrated. No trichinae were seen. The remainder of the gastro-intestinal tract, together with the spleen, pancreas, liver, gallbladder, biliary tract, and adrenal glands, showed no significant abnormalities.

The left kidney weighed 180 gm.; the right, 190 gm. The capsules stripped readily, revealing smooth reddish brown surfaces. At the

superior pole of the right kidney, several well circumscribed abscesses, 3 to 4 mm. in diameter, were present. The bladder was distended and the mucosa was decidedly hyperemic. Numerous small, confluent abscesses ramified through all lobes of the prostate gland. Many segments of the periprostatic veins contained ante-mortem thrombi. The urethra, seminal vesicles, and testes were not remarkable.

There was generalized osteoporosis of the skeletal system. The vertebral, costal, and femoral marrows were soft and reddish-gray, and certain areas suggested increased cellularity. No obvious neoplastic tissue was found, however, and no focal areas of bone destruction could be demonstrated.

There was mild generalized lymphadenopathy, most marked in the mesenteric nodes. Nowhere was the architecture distorted.

The brain weighed 1420 gm. On removing the intact dura, the leptomeninges were found to be covered with a rather thick, yellow, fibrinopurulent exudate which extended over the entire convexity of the cerebral hemispheres and the greater portion of the cerebellar hemispheres. The base of the brain was bathed in pools of pus, and the exudate was particularly conspicuous along the cerebral vessels and about the longitudinal fissure. There was flattening of the convolutions in the frontal and parietal areas. The lateral ventricles contained slightly cloudy fluid, but there was no displacement or dilatation of the ventricular system. The basal arteries showed moderate arteriosclerotic changes.

The thyroid, parathyroid, and pituitary glands were virtually normal.

Post-Mortem Laboratory Studies

Chemical examination of the blood: Nonprotein nitrogen, 282 mg. per cent; blood urea nitrogen, 192 mg. per cent; creatinine, 5 mg. per cent; ammonia, trace. Bacterial cultures of the heart's blood, lung, and brain: Luxuriant growth of *Staphylococcus aureus*.

Microscopic Examination

The muscle fibers of the heart were normal on microscopic examination. Scattered diffusely throughout the interstitial tissue of the myocardium were small nests of lymphocytes and mononuclear cells with occasional neutrophils and plasma cells. The inflammatory reaction, although minimal, was most conspicuous about capillaries. Occasional small areas of scarring were present. An occasional capillary was thrombosed. No larval or encysted forms of *T. spiralis* were found.

The pleural surfaces of the lungs were covered with thick masses of fibrin in which numerous areas of hemorrhage were present. The alve-

olar spaces were choked with acute inflammatory exudate. Areas of necrosis and abscess formation were present. The blood vessels were engorged and some were thrombosed. The bronchioles were filled with inflammatory exudate. Eosinophils were not especially numerous, but there were many focal clusters of plasma cells in the interstitial tissue. No trichinal larvae were found.

The architecture of the spleen was well preserved and the malpighian bodies were not hyperplastic. Sections from different parts of the organ revealed a diffuse infiltration of mature plasma cells throughout the red pulp. Frequently, these cells were found in clumps of 3 to 6, but nowhere were they replacing the normal cellular elements of the spleen. The reticulohistiocytic cells were increased in number, and many were swollen and granular. Both intracellular and extracellular deposits of hemosiderin were present. The sinusoids were distended with blood.

The entire gastro-intestinal tract was congested, and there was rather marked edema of the mucosa and submucosa, particularly in the small bowel. The lymphatic spaces were moderately dilated. There was considerable epithelial desquamation, but no ulcers were observed. In the lamina propria and the submucosa there were numerous plasma cells and lymphocytes, and occasional neutrophilic polymorphonuclear leukocytes. Eosinophils, although present, were not numerous. In many sections of the duodenum and upper jejunum, viable adult female forms of *T. spiralis,* some of them gravid, were present deep in the crypts of Lieberkühn and in Brunner's glands. The organisms were confined entirely to the mucosal and submucosal layers. None were found in the lymphatics, and there was no marked inflammatory response in the immediate vicinity of the parasites. The esophagus, stomach, and colon, except for minimal congestion, were unremarkable.

The renal tubules in some areas were moderately dilated. The tubular epithelium was intact, but many of the cells were swollen and granular. Clusters of acute inflammatory cells were observed in the tubules, and the interstitial tissue of the cortex and medulla, in some regions, was replaced by small confluent abscesses. Mild subacute inflammation of the mucosa and submucosa of the urinary bladder was present. Numerous confluent abscesses were scattered throughout the prostatic tissue. There was focal hyperplasia of glandular epithelium and fibromuscular stroma.

Sections of cervical and abdominal lymph nodes revealed distention of the lymphatic spaces with pink-staining protein material. Scattered indiscriminately throughout all portions of the nodes were numerous mature, normal-appearing plasma cells. Many were found singly, but

the majority occurred in clusters of 2 to 4 cells. When present in clusters, they appeared to be intimately associated with the reticulohistiocytic cells. As in the spleen and bone marrow, the plasma cells were arranged so haphazardly throughout the tissue as to suggest a diffuse hyperplastic process—decidedly not a neoplastic one. The reticulohistiocytic cells were increased in number, and frequently, swollen, granular forms were observed. The follicles were not enlarged, nor was there any evidence of a transition from lymphocytes to plasma cells.

Multiple sections from costal and vertebral marrows revealed rather marked plasmacytic hyperplasia. The plasma cells were intermingled with the other blood cells. Myeloblasts, myelocytes, and megakaryocytes were present in the usual distribution. There was a moderate increase in the number of normoblasts. Occasional clusters of from 5 to 8 plasma cells were observed, but by and large, the infiltration was diffuse, and nowhere did the cells assume neoplastic proportions. The bony trabeculae revealed no areas of destruction.

In Giemsa and phosphotungstic acid-hematoxylin preparations, the majority of cell types in the bone marrow were identifiable. With few exceptions, the plasma cells were of uniform diameter and were readily differentiated by their characteristic cytoplasmic and nuclear structure. The reticulohistiocytic cells were increased and frequently showed granularity and swelling. Although no attempt was made to ascertain the origin of the plasma cells, it was observed that in the spleen and lymph nodes as well as in the bone marrow plasma cells were generally most numerous in areas where reticulohistiocytic cells predominated. Furthermore, certain cytoplasmic and nuclear changes in many of the reticulohistiocytic cells suggested that the plasma cells took their origin from these cells.

Multiple sections from various skeletal muscles throughout the body revealed a large number of trichinal larvae, some nonencysted, but most in stages of encystment. Beginning capsule formation was observed in a few instances. The recently encysted larvae were mixed with older forms, the capsules of which were homogeneous and hyalinized. Partially calcified cysts were numerous. Calcification began at the poles of the lemon-shaped cysts, usually as fine, blue-black granules. Complete calcification was observed rarely. The local inflammatory response was minimal or absent except about the nonencysted larvae. Here, a foreign body reaction with moderate acute to subchronic inflammation was noted.

The leptomeninges over the cortical surface and at the base of the brain exhibited an extensive acute inflammatory reaction with abscess

formation. The subcortical brain tissue revealed small areas of hemorrhage and minute focal clusters of inflammatory cells and macrophages about the capillaries. No granulomatous nodules were observed and no trichinal larvae were found.

Sections of the liver, pancreas, adrenal, thyroid, parathyroid, and pituitary glands were unremarkable.

Final Diagnosis. Disseminated trichinosis; *Staph. aureus* septicemia; confluent bronchopneumonia with abscess formation; acute suppurative meningitis; benign prostatic hyperplasia with acute suppurative prostatitis; acute focal pyelonephritis; subacute myocarditis; diffuse plasma cell hyperplasia; hyperglobulinemia; hypochromic, microcytic anemia; diffuse osteoporosis; uremia, terminal.

Discussion

The case presented is that of an infection with *T. spiralis,* complicated terminally by *Staph. aureus* septicemia, pyemia, and uremia. The striking rouleaux formation, hypochromic anemia, and hyperglobulinemia, together with other suggestive clinical manifestations, supported the tentative clinical diagnosis of multiple myeloma. In addition to the plasma cell response and the sequelae thereof, certain clinical and pathologic features of trichinosis as related to the present case require comment.

At autopsy a small piece of grossly infected pectoralis major muscle was crushed between two glass slides and examined microscopically. Nonencysted trichinae exhibited their rather characteristic spiral motion, and excystment of some larvae with subsequent curling and uncurling was observed. A quantitative estimation of the infection was ascertained with the use of the trichinoscope. Since it is sometimes difficult to detect noncalcified encysted and nonencysted trichinae because of their transparency, the skeletal muscle was fixed in 10 per cent formalin. The fixation increases the opacity of the parasites and their capsules, and thereby greatly facilitates their detection. The formalin-fixed tissue was then dried, and 1 gm. portions were teased apart and placed between the glass plates of the trichinoscope. The results of two of the counts are shown in Table I.

If one disregards those trichinae in which the capsules were completely calcified, then the degree of infection, as determined by Hall and Collins,[10] may be considered to be moderate to heavy. This in itself, however, means little with regard to possible fatal outcome or even clinical manifestations. Gould,[11] for example, has found trichinous infection of 101 to 1000 larvae per gm. of muscle at autopsy in each of

11 persons who died from diseases other than trichinosis. Furthermore, in none of these 11 persons was there a clinical history of trichinosis. It has been repeatedly observed that many variables such as size and age of the patient, nutritional status, and the presence of other concomitant morbid processes in the body may ultimately determine the subsequent sequence of events and the end result. Nevertheless, the fundamental disease process in this case may be regarded, justifiably, as one of trichinous infection.

In regard to the biologic history of *T. spiralis* as it pertains to this case, it should be emphasized that adult female worms, some of them gravid, were found in the mucosa of the duodenum and jejunum. It is generally believed that following copulation, the male intestinal trichina

TABLE I

Incidence of Larval Forms in Skeletal Muscle

Larval form	Diaphragm	Psoas
Nonencysted	8	5
Encysted	76	25
Beginning calcification of capsule	58	26
Complete calcification of capsule	2	5
Total	144 per gm.	61 per gm.

dies and is digested, and that following the birth of the larvae, the female intestinal trichina likewise dies and is digested.[12] According to Cameron as quoted by Faust,[13] the young females are fertilized on about the 3rd day after ingestion of infected meat, and begin to larviposit on the 5th day. Larvipositing decreases by the 14th day. Of more significance, however, is the fact that the maximum life span of the mother worms in the intestinal wall is not known with certainty. In the case presented, if we date the length of the disease from the onset of the respiratory symptoms, then viable adult female intestinal trichinae were present for 115 days. If we accept the general observation that respiratory symptoms develop 2 to 6 days following the ingestion of infected meat, then the adult female trichinae were present for approximately 120 days. Assuming that the severe nonproductive cough (4 weeks prior to admission) was totally unrelated to the trichinous infection, the patient was, nevertheless, in the hospital under close observation for a total of 87 days. There is, of course, the possibility of reinfection. If so, the source might have been the hospital food, a fact which has not been overlooked. Meat was carefully inspected, and nothing was found to incriminate it as a possible source. Furthermore, no cases of clinical trichinosis were

found in the hospital personnel or in the other 1100 patients who ate similar food at all meals. The patient was too ill to subsist outside the hospital, and as far as could be determined, no food was sent to him from the outside. It would seem most probable, therefore, that the infection was acquired before entering the hospital, in all probability approximately 5 weeks before admission. The presence of nonencysted motile larvae in muscle also proves that larvipositing can occur for at least 4 months. Since the overwhelming majority of larvae counted were either encapsulated or showed beginning calcification of the capsules, and since encapsulation begins about the 21st day of the infection, and beginning calcification of the capsules at about 6 months,[13] it would seem quite possible that the patient consumed infected meat on more than one occasion. Furthermore, it would appear that the greatest number of trichinae were consumed about 5 months apart. And yet in view of finding adult female intestinal trichinae and nonencysted larvae in muscle 4 months from the onset of the illness, it would appear equally possible that the infection could have been acquired after just one exposure. The presence of a few larvae with complete capsular calcification, a process which is believed to require about 1 year at least, indicates that these must have been derived from a previous infection.

In an attempt to integrate the clinical findings as related to trichinosis, it will be noted from the history that the onset of illness 4 weeks prior to admission was characterized by a moderately severe, nonproductive cough which lasted 5 days. This symptom, although usually not a serious one, is present in approximately 30 per cent of cases and frequently marks the onset.[14] The cough is generally attributed to the passage of larvae through the pulmonary capillaries in their transit from the intestinal mucosa to the peripheral circulation.

One week following the onset of the cough, pain in the muscles of the back made its appearance. It is not unusual in trichinosis for pain to be limited to one or two muscle groups such as the back and thigh,[15] and it occurred at a time when the migration of larvae to skeletal muscle might be anticipated. Of the muscles of the back, the lumbar group are apt to be the most heavily seeded.[16] The pain gradually increased in severity, and although aggravated by both active and passive motion, it was present also at rest—findings quite in keeping with the character of muscle pain in trichinosis.

The absence of certain clinical manifestations which are so commonly a part of trichinosis is of considerable interest. There were no abdominal symptoms. Ocular movements elicited no pain, and there was no edema, generalized or local. Fever was not present terminally, nor were

there chills, sweats, or malaise. Hypochromic anemia was moderate, and eosinophilia (15 per cent) was found on the admission count only. The absence of these manifestations, however, does not preclude the diagnosis of trichinosis.

Although myocarditis constitutes the chief danger in trichinosis, and subsequent congestive heart failure is the most common cause of death, the subacute myocarditis in this case was minimal and probably contributed only slightly, if at all, to the patient's death. Septicemia is considered a rather rare complication of trichinosis,[17] but *Staph. aureus* septicemia, pneumonia, meningitis, and pyelonephritis were the terminal events in this case.

The Plasmacytic Reaction

The presence of numerous plasma cells in the bone marrow, lymph nodes, and spleen was a thought-provoking finding. Was the plasmacytic response an unusual manifestation of trichinosis, or was a concomitant multiple myeloma or myelomatosis developing? The indiscriminate distribution of typical mature plasma cells among the normal tissue cells, rather than the presence of obvious aggregates of these cells, is evidence in favor of the former. The absence of discrete skeletal defects, tumor formation, and other pertinent clinical manifestations frequently observed in multiple myeloma argues against this diagnosis. Yet when one considers the clinical and pathologic variations that may occur in the group of myelomatous diseases, particularly in the early stages, there remains considerable doubt concerning the true nature of the plasma cells in this case.

A variant of the plasma cell tumors, the so-called generalized or diffuse myelomatosis, has been described particularly by the French and Scandinavian writers.[18–21] It is a common experience to find that with multiple discrete plasma cell tumors there is, to a greater or lesser extent, a diffuse distribution. But a diffuse malignant plasma cell invasion of the skeleton and some viscera without evidence of focal neoplastic masses must be quite rare. Geschickter [22] has yet to observe such a case. Marchal and Mallet,[20] on the other hand, have reported a case in which there was extensive diffuse plasma cell invasion of the skeletal system. The roentgenograms of the skeleton revealed diffuse radiolucency without cysts, erosions, or punched-out areas. Their patient exhibited no tumors nor Bence-Jones protein, but did maintain hyperglobulinemia and moderate hypochromic anemia. These authors were convinced that their case was one of plasma cell myeloma. Their findings are comparable to those in the present case in respect to plasmacytosis. However,

some of the reported cases of diffuse myelomatosis are of doubtful authenticity as to being fundamentally neoplastic. Tuberculosis, as well as certain other chronic diseases in which there is frequently a proliferation of plasma cells and hyperglobulinemia, cannot be excluded entirely.[19] Furthermore, there is often considerable doubt as to the significance of the bone changes on roentgenographic examination. In the vast majority of reported cases of diffuse myelomatosis, the patients were in an age group in which the changes of senile osteoporosis might be expected to occur.

A detailed study of the structure of the plasma cells in this case was of no practical value in determining whether the cells were fundamentally neoplastic. The majority appeared mature and similar in all respects to the plasma cells commonly found in chronic inflammatory lesions. Even in the group of extramedullary plasmacytomas, it is often impossible to determine whether an individual tumor is benign or malignant. As Hellwig,[23] in his general review of the subject, aptly pointed out, the microscopic appearance of the plasma cell cannot be relied on for prognosis.

When we consider the more common plasmacytic response which results from certain injurious agents, we find many diseases in which this cell is an outstanding feature of the cellular reaction. Many chronic and granulomatous infections such as tuberculosis, syphilis, sarcoid, leprosy, lymphogranuloma inguinale, bronchiectasis, pyonephrosis, rheumatoid arthritis, kala-azar, filariasis, and schistosomiasis, are characterized by proliferation of plasma cells during some stage of their course. In none of these diseases is there anything particularly remarkable about the morphologic characteristics of these cells. Quantitatively, they vary with the etiologic agent, the severity of the infection, and the general resistance of the tissues. It is common knowledge, too, that hyperglobulinemia frequently is associated with the above entities, at least during some phase of the disease process.[24-26] The hyperglobulinemia is seldom, if ever, as marked as with malignant myelomas, but neither is the plasma cell response so extensive or diffuse. Many of the above diseases are characterized by involvement of the reticulohistiocytic system as well as by proliferation of plasma cells. This fact supports the theory that some serum protein is formed by the plasma cells and the cells of the reticulohistiocytic system,[24,26-30] thus stressing the functional relationship between the two types. The findings in the case presented are in accord with the above statement in this regard.

So far as I am aware, there has been no other report of a plasma cell response with associated hyperglobulinemia in a case of trichinosis.

Thus, although a definitive statement concerning the fundamental nature of the plasma cell response in the present case cannot be made, all factors considered, it appears highly probable that the plasma cells constitute a primary cellular response to trichinosis.

Summary

Viable adult trichinae, including gravid females, were observed microscopically in the small intestine of a fatal case of human trichinosis 115 days after the onset of symptoms. The patient was continuously hospitalized and under close observation for 87 days. Reinfection, although remotely possible, was considered to be adequately excluded. The period of persistence of living adult trichinae in the human intestine is the longest thus far recorded. The continued release of larvae over relatively long periods of time is of considerable significance from a clinical, prognostic, and therapeutic point of view. The degree of infection as determined by counts with the trichinoscope was considered moderate to heavy.

The diffuse plasmacytosis with associated hyperglobulinemia is considered to constitute an unusual, if not unique, cellular response to trichinosis.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 42

FIG. 1. Adult trichina in mucosa of jejunum. × 100.

FIG. 2. Nonencysted larva in pectoralis major muscle. × 100.

FIG. 3. Encysted larva in pectoralis major muscle. Of note are the muscle nuclei within a cyst. × 100.

FIG. 4. Encysted larva in pectoralis major muscle, showing partial calcification. × 100.

FIG. 5. Plasma cell infiltration in vertebral bone marrow. × 200.

FIG. 6. Plasma cell infiltration in lymph node. × 400.

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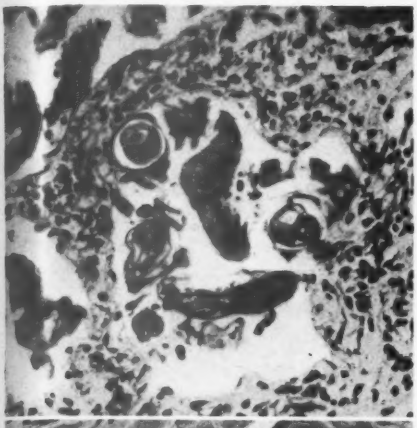

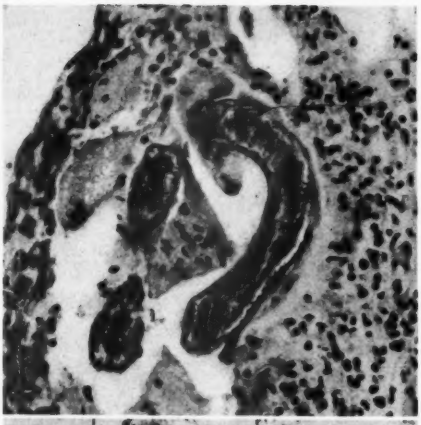

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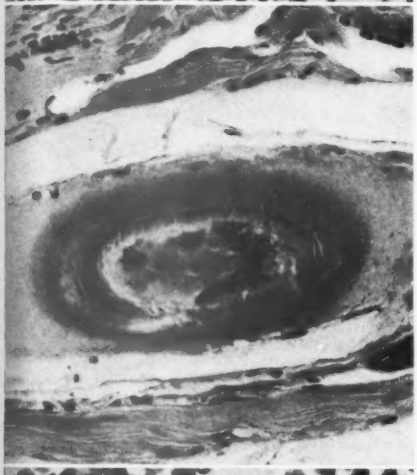

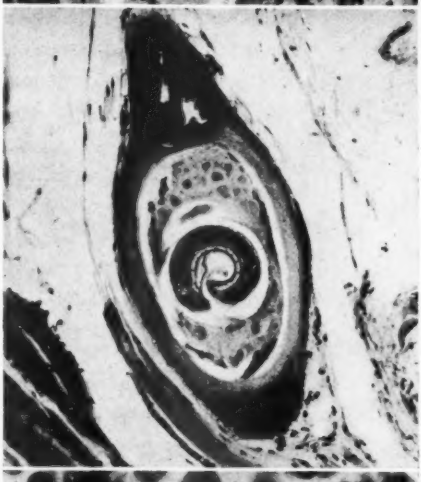

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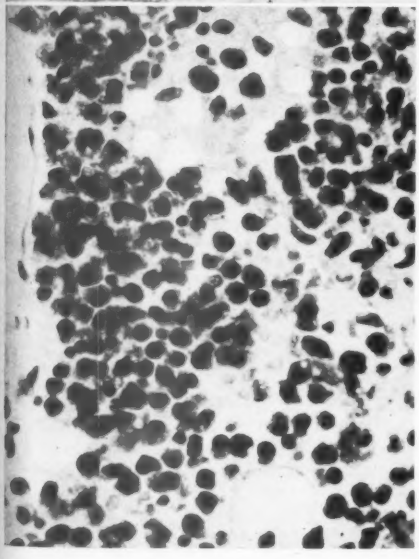

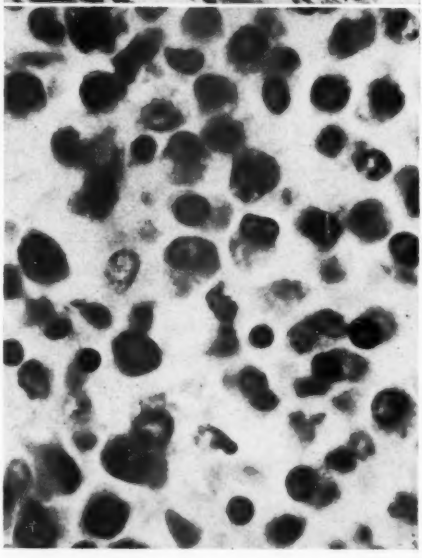

6

Carter

Plasma Cell Hyperplasia in Trichinosis

DIVERTICULA IN THE TERMINAL PORTION OF THE COMMON BILE DUCT *

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Standard textbooks describe the termination of the biliary tract almost exactly as it was done by Vater,[1] Opie,[2] Oddi,[3] and others [4] prior to 1905. Recent studies by Dardinski,[5] Michels,[6] and Schwegler and Boyden [7] have not been included in the standard descriptions of the anatomy of the biliary tract. Surgical procedures on the common duct have increased in scope and incidence; hence there is a stimulus for more specific information regarding its termination. My studies of fresh operative and autopsy specimens have been supplemented by injection of gelatin, dyes, and radio-opaque materials, as well as by detailed dissections in formalinized specimens. An abnormality found during investigations on the terminal portion, or pars intestinalis,[4] of the common bile duct is reported.

Four diverticula of the terminal or intrapapillary † portion of the common bile duct were observed in a series of 70 anatomic dissections. A fifth specimen in this series contained a choledocho-pancreatic duct fistula. These morphologic variations have not been reported previously.

METHOD

The duodenum, head of the pancreas, the hepatic pedicle, and adjacent tissues were removed at autopsy. In certain specimens air was injected into the duodenum after clamps had been applied to both ends. In a few specimens, diodrast or lipiodol was injected into the common bile and pancreatic ducts and radiographs were taken.

Specimens were preserved in a 4 per cent solution of formalin for 48 to 72 hours prior to complete dissection. The intact common bile duct was exposed throughout its extraduodenal course. Overlying pancreatic tissue was incised from the duodenum toward the duct. The proximal portion of the papilla was identified at the duodenal wall, and dissection was continued in order to expose the pancreatic duct as it emerged from the head of the pancreas. With fine-pointed scissors the lateral aspect of each duct was opened and the duodenal canal was entered through the papilla. The walls of the ducts were everted for inspection. Sections were cut for microscopic examination, particularly of the pancreas, and these were examined in consultation with Dr. Helen Ingleby.

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† The term *papilla* is used by Dardinski [5] and is the anatomic equivalent of the "pars intestinalis" of the common bile duct as described by Boyden.[4]

TABLE I

Measurements of the Extraduodenal Segment of the Common Bile Duct

Diameter of the lumen		Thickness of wall	
Number of cases	Diameter (mm.)	Number of cases	Thickness (mm.)
3	3.2	16	0.4
5	4.0	22	0.8
9	4.8	2	1.2
1	5.2	2	1.6
1	5.6	Total 42	0.7 (Average)
13	6.4		
4	7.2		
5	9.6		
1	10.4		
2	11.9		
1	12.7		
Total 45	6.4 (Average)		

TABLE II

Measurements of Diameter of Extraduodenal Common Bile Duct on Cholangiograms

Total series		Clinically normal patients	
Number of cases	Diameter (mm.)	Number of cases	Diameter (mm.)
3	4	3	4
2	5	2	5
7	6	7	6
5	7	4	7
2	8	2	8
4	9	2	9
4	10	4	10
2	11	Total 24	6.9 (Average)
5	12		
3	13		
1	14		
1	15		
4	16		
0	17		
0	18		
1	19		
0	20		
2	21		
3	22		
1	23		
Total 50	11.1 (Average)		

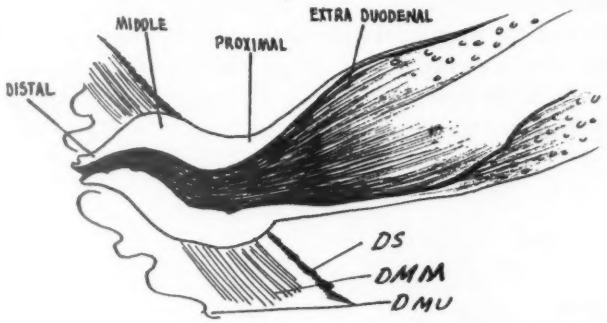
RESULTS

A total of 70 specimens were dissected. In a series of 45 formalinized specimens, the wall of the common bile duct was found to have an average diameter of 6.4 mm., and to be 0.7 mm. thick in its extraduodenal course (Table I). The extraduodenal diameter of the common bile duct as seen on the cholangiogram averaged 6.9 mm. in the normal patient (Table II). In the gross specimen and on the cholangiogram it was observed that the normal diameter of the common bile duct was less than 1 cm. Differences in measurement between fresh and formalinized specimens were carefully studied and it was found that an error up to 5 per cent was possible.

NORMAL STRUCTURE

On the radiograph taken after lipiodol was injected into the pancreatic duct and diodrast into the common bile duct, it was evident that each duct emptied separately into the duodenum (Figs. 2 and 3). The lumen of the common bile duct decreased in diameter as the duct crossed the duodenal wall.

Examination of the dissected specimen revealed a difference between the extraduodenal and transduodenal (pars intestinalis) segments of the



Text-Figure 1. Sketch indicates the major divisions of the common bile duct as (A) extraduodenal, and (B) transduodenal or pars intestinalis, which, in turn, is divided into proximal, middle, and distal portions from without to within the duodenum. DS = duodenal serosa; DMM = duodenal musculature; DMU = duodenal mucosa.

common bile duct (Text-Fig. 1). The extraduodenal portion had a wide lumen, a thin wall, and a smooth mucosa which was stippled by the orifices of numerous glands.[5] In the transduodenal segment of the common duct, the lumen became a funnel which decreased in diameter to terminate as a narrow filamentous canal. The wall of this segment was thick and the mucosal lining had many reduplications and valvules.[2,5,8] It was in the valvular area of the common bile duct that four diverticula and one fistula were observed.

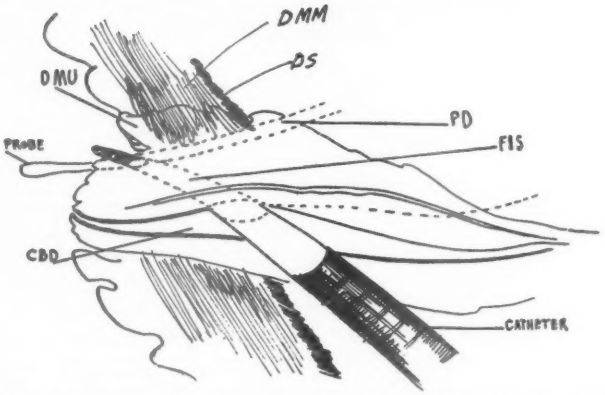
OBSERVATIONS ON ABNORMALITIES

Specimen 41. In specimen 41, the diameter (6.4 mm.) and the thickness (0.8 mm.) of the wall of the extraduodenal portion of the common duct were normal. In the distal half of its pancreatic course the common bile duct was covered with pancreatic tissue. The pancreatic and common bile ducts opened through separate orifices into the duodenum. The papilla was 12.8 mm. long and its wall averaged 3.4 mm. in thickness. Four large valvules and many smaller reduplications were present

in the transduodenal segment of the common duct. Beneath a cusp of a valvule in the middle third of the intrapapillary portion of the common bile duct, a 2.0 mm. orifice admitted a probe into a tract which extended approximately 1.5 cm., to empty into the pancreatic duct 2.0 mm. from the termination of the duct in the duodenum (Text-Figure 2). The fistula was empty. There were no other abnormalities of the ducts, pancreas, or of adjacent organs.

This choledocho-pancreatic duct fistula was probably a developmental anomaly.

Specimen 25. The lumen (4.8 mm. in diameter) and wall (0.8 mm. in thickness) of the extraduodenal segment of the common bile duct in specimen 25 were normal in size. The common duct was intrapancreatic for one-third of its pancreatic course. The common bile and pancreatic ducts emptied through the papilla into the duodenum by separate orifices.



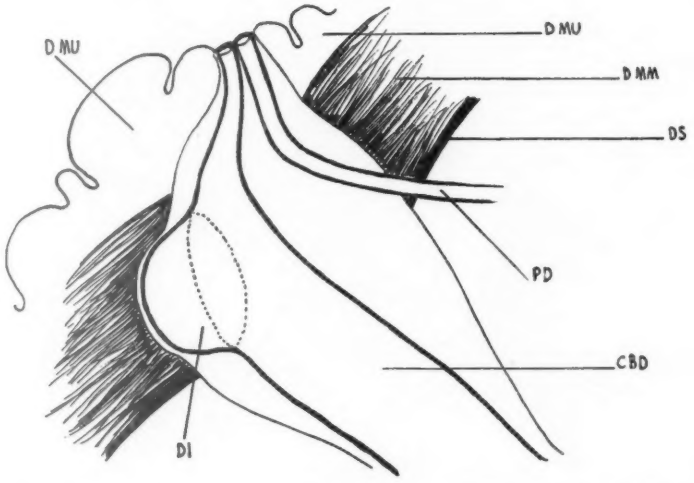
Text-Figure 2. A catheter is passed through the choledocho-pancreatic duct fistula (FIS). A probe is in the pancreatic duct (PD) emerging through the orifice into the duodenum. The orifices of the common bile and pancreatic ducts are separate. CBD = common bile duct. (Specimen 41. × 5.)

The papilla was 18.9 mm. long and its wall was 2.4 mm. thick. There were many valvules in the papillary portion of the common duct. An area in the proximal half of the papilla was less than 1.0 mm. thick. Here a diverticulum was found emerging toward the duodenum and impinging on the duodenal wall (Text-Fig. 3). There were no calculi in the common bile duct. The pancreas was normal grossly.

Specimen 40. In specimen 40, the diameter (10.4 mm.) and thickness (1.2 mm.) of the wall of the common bile duct in its extraduodenal portion were slightly greater than normal. In its pancreatic portion the common bile duct was completely surrounded by pancreatic tissue. The pancreatic and common bile ducts opened through separate orifices into

the duodenum. The papilla was 14.3 mm. long and the thickest portion of its wall measured 4.0 mm. There were many valvules in the intrapapillary portion of the common bile duct. In the middle third of the papilla an orifice approximately 2.0 mm. in diameter was observed through which a diverticulum had opened. The sac was approximately 6.0 mm. long. It contained three small, black calculi and some bile-stained débris (Text-Fig. 4).

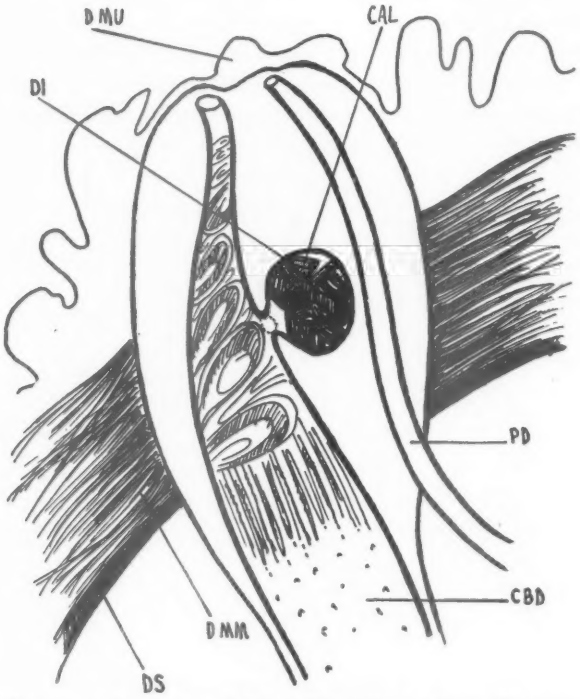
Grossly, the pancreas was firm and sclerotic. Microscopically, there was recent necrosis of the parenchyma with evidence of fatty infiltration and fat necrosis. There was severe periacinar fibrosis and minimal cellular reaction.



Text-Figure 3. A diverticulum (DI) is present in the proximal segment of the papilla. The diverticulum extends toward the duodenal wall and impinges on the serous and muscular layers. There are separate orifices for the common bile and pancreatic ducts. (Specimen 25. × 5.)

Specimen 63. The patient was a white male, 63 years old, who was treated surgically on the service of Dr. Ralph Goldsmith (Jewish Hospital, Philadelphia), for a pancreatic pseudocyst and recurrent pancreatitis. The pseudocyst was drained. Several months later, cholecystectomy was performed because of cholelithiasis. The common duct was normal grossly and no calculi were found. A "T" tube was sewn into the common duct. A cholangiogram was made on the 15th postoperative day. The hepatic ducts were not dilated. There was extravasation along the course of the "T" tube. Dye entered the duodenum rapidly. There was a small irregularity in the distal portion of the common bile duct. Less than 1 cm. from the termination of the com-

mon bile duct the distal portion of the pancreatic duct was demonstrated, and both ducts had a common orifice extending through the innermost portion of the duodenal wall (Fig. 1). Massive bleeding through and around the "T" tube followed ½ hour after cholangiography, subsiding spontaneously. The "T" tube was removed, and the patient discharged.



Text-Figure 4. A diverticulum of the medial portion of the papilla is seen to emerge on the pancreatic aspect and to penetrate toward the pancreatic duct. CAL = calculi in the diverticulum. (Specimen 40. × 5.)

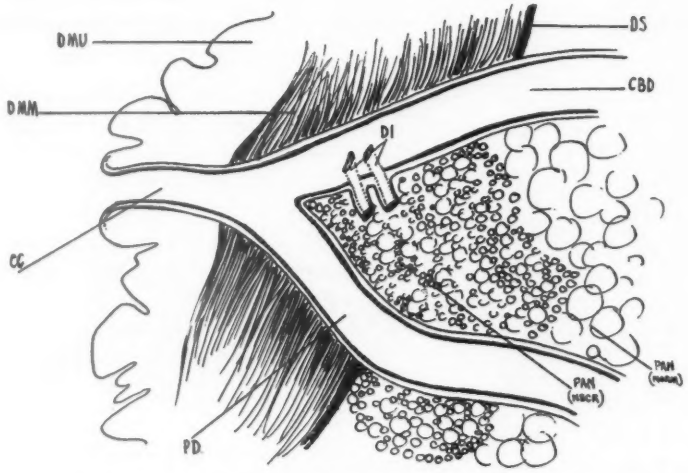
Intermittent bleeding continued. The patient was readmitted to the hospital 4 months later because of hematemesis, melena, a marked secondary anemia, jaundice, and severe hepatocellular damage. Two months later, he died of exsanguination.

The cause of death was found to be bleeding from an erosion of the right branch of the hepatic artery into the common bile duct. This was probably secondary to the action of fluid leaking from the pancreatic pseudocyst.

On post-mortem examination of the biliary tract, the lumen (5.0 mm. in diameter) and wall (0.8 mm. in thickness) of the extraduodenal common bile duct were normal. The pancreatic duct joined in a com-

mon channel with the common bile duct; this common channel was 6.0 mm. long and extended through the duodenal mucosa.

The papilla was 15.0 mm. long; its wall was less than 1.5 mm. in thickness throughout. Papillary tissues were atrophied; only flattened markings and valvular remnants were observed in the intrapapillary segment. Calculi were not found.



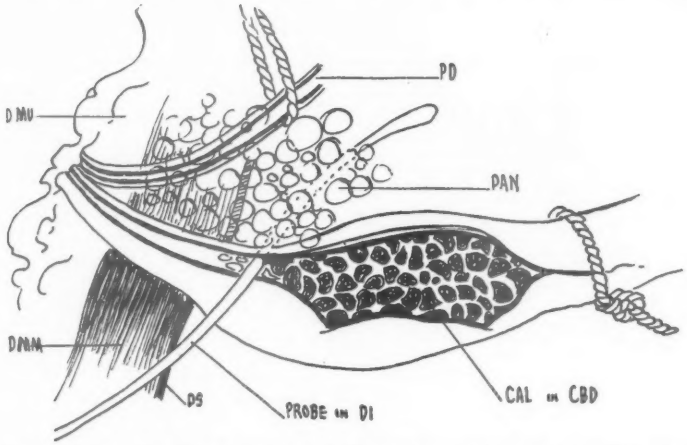
Text-Figure 5. Two diverticula pass from the common bile duct to terminate within an area of pancreatic necrosis (PAN, NECR). The common channel (CC) for the common bile and pancreatic ducts is distal to the location of the diverticula (DI). (Compare Figure 1.) (Specimen 63. × 5.)

In the middle third of the intrapapillary portion of the common bile duct and proximal to the beginning of the common channel for both ducts, two oval orifices were observed, each with a diameter of 1.0 mm. (Text-Fig. 5). A probe was passed through these openings into two small sacs which extended into pancreatic tissue for 5.0 and 8.0 mm., respectively. The sacs were closed, although their walls were friable. Pancreatic tissue adjacent to the diverticula was necrotic for a radius of 2.5 cm. The body of the pancreas was sclerotic. The main pancreatic duct was dilated. In the body of the pancreas, approximately 6.0 cm. from the papilla, a sinus tract extended from the main pancreatic duct into a large cavity which had dissected the layers of the gastrohepatic omentum to form a pseudocyst.

Microscopically, the pancreas showed severe chronic pancreatitis with necrosis, diffuse inflammatory exudate, and periacinar and perilobular fibrosis.

Specimen 36. The diameter (6.4 mm.) and width of the wall (0.8

mm.) of the extraduodenal common bile duct of specimen 36 were normal. The duct was intrapancreatic for the distal half of its pancreatic course. The pancreatic and common bile ducts emptied separately into the duodenum. The papilla was 17.0 mm. long and its wall was 6.4 mm. thick. Valvules in the transduodenal segment of the common bile duct were thicker than is normal. On the pancreatic aspect of the papilla a thin, narrow diverticulum was found which was 8.0 mm. long and 1.5 mm. wide (Text-Fig. 6). This diverticulum contained



Text-Figure 6. The probe has entered a diverticulum originating in the proximal portion of the pars intestinalis of the common bile duct. The diverticulum ends with adjacent pancreatic tissue. (Specimen 36. × 3.)

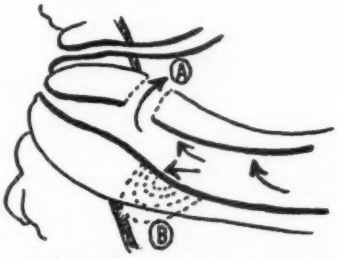
small calculi. The common bile duct contained many faceted gallstones. There was another area in the proximal portion of the papilla which, because of the extreme thinness of the wall, may have represented the primary stage in the formation of another diverticulum. Adjacent pancreatic tissue was sclerotic grossly. Microscopically, there was slight increase in perilobular fibrous tissue, with moderate edema, early degeneration, and necrosis present in a few acini.

Discussion

Obstruction to the flow of bile from the distal termination of the common bile duct may be produced by calculus, stricture, spasm, inflammation, or neoplasm. Increased intracholedochal pressure frequently occurs under such conditions, so that the normal pressure of 10 to 15 cm. of water may be increased to 60 to 75 cm. of water.[9,10] When increased pressure is maintained, and particularly if a calculus is present

and/or if there is a defect in the continuity of the duct, these may summate to provide an alternate route of least resistance for the passage of bile from the common duct into a diverticulum, and eventually a choledochal fistula might form.

In the distal centimeter of the common bile duct, the lumen is decreased and the wall is thicker. A stone is normally impacted proximal to these thickened tissues of the common duct papilla and the narrow filamentous portion of its lumen. There would be a tendency for a stone to erode through the thinner tissues toward the pancreas and its duct (Text-Fig. 7, A), or toward the duodenal wall (Text-Fig. 7, B) to form a choledocho-duodenal fistula. A pre-existing structural deficiency and the trauma and inflammation associated with choledocholithiasis could well be the etiologic factors in the pathogenesis of diverticula in the papillary region of the common bile duct.



Text-Figure 7. The diagram indicates potential directions taken by diverticula formed when increased intracholedochal pressure is exerted against the papilla. In "A" the diverticulum penetrates toward the pancreatic duct and in "B" toward the serosa of the duodenal wall.

Two of four reported diverticula contained biliary calculi. Pancreatitis was present in these cases. A third case with diverticula had a past history of pancreatitis and of a pancreatic pseudocyst. In that case, cholecystectomy was done for cholelithiasis. However, on final examination choledocholithiasis could not be demonstrated. In the fourth specimen of a diverticulum there was neither evidence of pancreatitis, nor were calculi found in the common duct, gallbladder, or pancreas.

Calculi in a diverticulum may be asymptomatic. The stone and diverticulum might escape observation on the postoperative cholangiogram if the orifice of the sac was occluded by the stone or by edema. On the other hand, the escape of bile or of a calculus through such a diverticulum into adjacent tissues might be manifest clinically as an episode of pancreatitis. This becomes a means for the introduction of bile into the pancreas.[11-19] When pancreatitis is evident, specimens of the distal segments of the common bile and pancreatic ducts should be carefully examined after preservation in formalin. Unless their existence is suspected and observation directed toward their demonstration, diverticula in the terminal portion (pars intestinalis) of the common bile duct can be overlooked.

Conclusions

Abnormal channels in the terminal portion of the common bile duct were found in 7 per cent of a series of 70 anatomic dissections.

Four diverticula and one choledocho-pancreatic fistula are reported. Two diverticula contained stones. In these two specimens there was gross and microscopic evidence of pancreatitis.

One patient with diverticula of the common bile duct had a history of pancreatitis and cholelithiasis. There were no calculi demonstrated in the common duct nor in the diverticula at the time of examination of the specimen.

Diverticula of the common bile duct may be a factor in the pathogenesis of certain forms of pancreatitis.

A diverticulum in the terminal portion (pars intestinalis) of the common bile duct is easily overlooked. It is recommended that careful dissection of the formalinized specimen be done in cases presenting a history of pancreatitis.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 43

FIG. 1. On this indirect cholangiogram diodrast entered the duodenum without obstruction to its flow. There is a concavity of the common hepatic and bile ducts due to pressure by a pancreatic pseudocyst. The medial convexity of the distal segment of the common bile duct terminates as a small S-shaped curve.

The last centimeter of the pancreatic duct as well as 7 mm. of a common channel for the pancreatic and common bile ducts is visualized.

FIG. 2. Diodrast injected through the catheter in the common bile duct entered the duodenum through a funnel-like lumen which terminated as a filamentous canal.

FIG. 3. Lipiodol injected through a needle into the pancreatic duct diffused through the pancreatic ductal system. It emptied into the duodenum alongside the common bile duct, but through a separate orifice.

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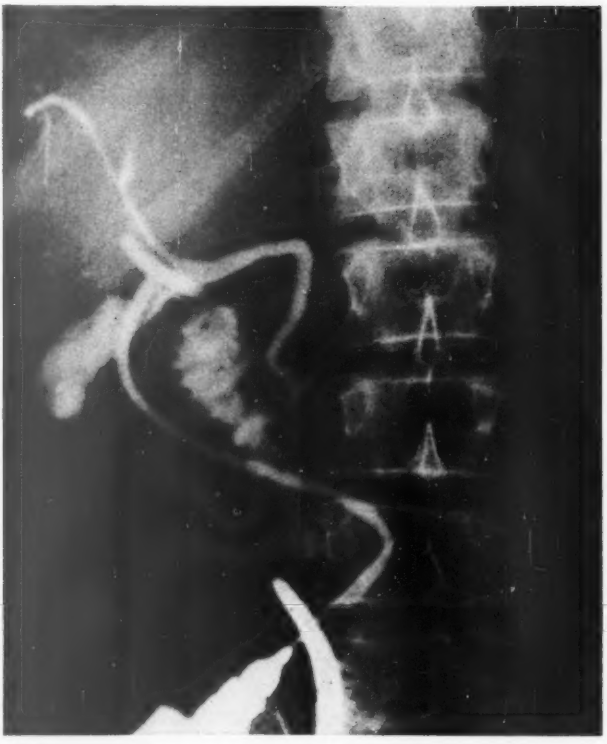

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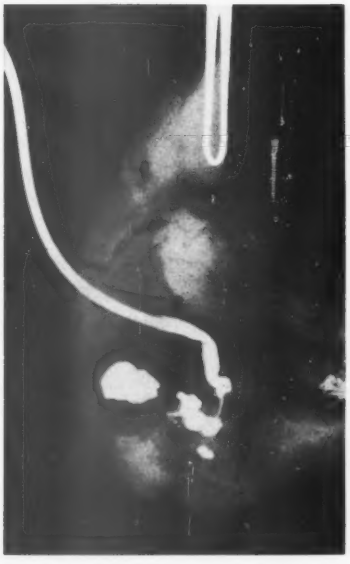

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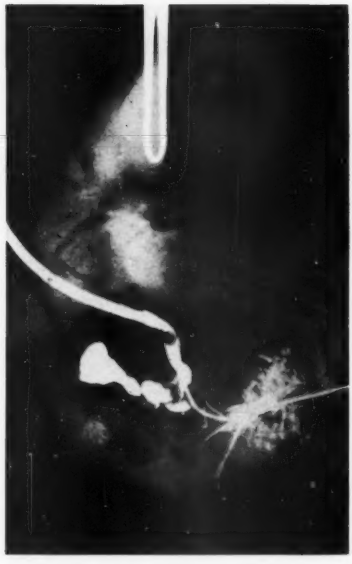

NEOPLASTIC DISEASES OF DOGS

I. Neoplasms of Melanin-Forming Cells *

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Among the papers in a recent symposium [1] devoted exclusively to a consideration of melanoma, no example of a neoplasm of melanin-forming cells in dogs was recorded. The scarcity of melanoma in the dog is attested by the relatively few cases in several compilations [2-13] of tumors in dogs found in the literature. For this reason an analysis has been made of the melanin-containing neoplasms of the skin of 31 dogs, with regard to their gross and microscopic features. These have been correlated with available information on breed, age, sex, sites of predilection, and some clinical details.

Review of Literature

In an extensive survey of the early literature of tumors in animals, Casper [2] found 3 instances of melanoma in dogs, the first observed by Kitt [3] in the neck, the second by Bruckmüller [4] at the base of the brain as small tumors, and the third by Bournay [5] in the mouth, shoulder, heart, and lungs as multiple sarcoma, partly melanotic and partly telangiectatic. McFadyean [6] noted a "melanotic carcinoma" of the skin of the back among 23 cases of carcinoma in dogs. Of 26 cases of canine sarcoma, Murray [7] found "melanotic sarcoma" of a hind leg in 2 dogs. Although he had a wide personal experience with tumors in dogs, Joest [8] recorded but one instance of melanoma, a "melanosarcoma" of the nose with metastases in the lungs.

In a review of 2499 histologically confirmed canine neoplasms, Fölger [9] found but 3 "melanosarcomas." Among 31 multiple primary tumors in dogs, Cohrs[10] observed, adjacent to the left eye of a 14-year-old male dachshund, a cutaneous melanoma with transformation to spindle cell sarcoma. A "melanosarcoma" of a mammary gland, with metastases in the brain and spinal cord, was described by Feldman [11] in a group of 17 canine tumors. Among 15 dogs with microscopically verified neoplasms, Chambers [12] reported the case of a 10-year-old male Aberdeen terrier with "melanotic sarcoma" of the inguinal region. Of the 336 histologically examined canine tumors in the large series of Auler and Wernicke,[13] only one was a "melanosarcoma." Passey [14] was

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able to produce malignant melanoma in the dorsal cervical region of 2 mongrel Airedale bitches by applying tar weekly for about 7 years.

McClelland [15] mentioned a melanoma in a young male Doberman-Pinscher examined by Fehr and Wilder.[16] He also described 2 cases of his own. The first was an 8-year-old male chow which had a malignant melanoma of the metacarpal region with metastases to popliteal, inguinal, and mesenteric lymph nodes and to the lungs. The second concerned a 10-year-old male chow with a malignant melanoma of the tongue which metastasized to cervical lymph nodes, mediastinum, pleura, and lungs. He also had seen 2 aged Boston terriers each afflicted by melanoma of the choroid of one eye.

Bloom [17] recorded the case of a 1½-year-old male chow which had a "melanocarcinoma" of the iris of the right eye.

Definition of Terms

The term "noncancerous melanoma" will be employed to designate the benign neoplasm of cells capable of elaborating pre-melanin (melanoblasts) or melanin (melanocytes). The term "nevus" is objectionable, since it means simply a "blemish," usually of congenital origin, and may be derived from endothelial cells, pigment-forming cells, or squamous epithelial cells. "Melanocytoma" is inappropriate, for this name may include all cells containing melanin, such as macrophages and the cells of the bottom row of the epidermis. Although melanoma has been used to mean a "malignant" neoplasm of cells capable of forming melanin within themselves, this is to be deplored as much as if fibroma or chondroma were similarly abused.

The term "cancerous melanoma" will be used to designate the anaplastic, invasive, and metastasizing neoplasm derived from cells capable of forming melanin. "Cancer" has been established by long usage as a name for neoplasms of any type which are "malignant" or "nonbenign" or anaplastic, invading, and metastasizing, although some still restrict its use to designate an anaplastic epithelial growth, namely, carcinoma. Consequently, "malignant" has been avoided, even though it means "acting maliciously." The designations "melanocarcinoma" and "melanosarcoma" have been shunned. Usually these terms simply indicate that polyhedral cells or spindle cells preponderate in a given cancerous melanoma, whereas mixtures of the two in varying proportions are often found when enough sections of the primary neoplasm and/or its metastases have been examined. Some workers believe that melanoblasts, the basic cells of "melanoma," are of ectodermal origin, whether through migration of neural crest cells into the skin or from

primary origin in the epithelial cells of the epidermis. Thus, "melanocarcinoma" might be suitable, but gliomas and nerve sheath tumors should then be grouped under "neurocarcinoma," an obviously objectionable procedure. If the term "melanosarcoma" were used, then the evidence supporting an ectodermal origin for melanoblasts must be disregarded. However, this term might be acceptable when the close relation between the embryonic development of nerve sheath cells and melanoblasts is taken into account, for the cancerous variant of the nerve sheath tumor (neurofibroma) may be properly labeled "neurofibrosarcoma."

Materials and Methods

The neoplasms examined were obtained from several veterinarians.* The tissues were surgically resected in 22 cases, were obtained at reasonably complete autopsy in 7 cases, and were excised after death in 2 cases. They were fixed in 4 per cent formaldehyde and routine tissue sections, stained by hematoxylin and eosin, were prepared as previously described.[18] Becker's silver nitrate stain [19] for melanin and Turnbull's blue reaction for iron-containing pigment were used on several neoplasms. A few heavily pigmented lesions were bleached by potassium permanganate and oxalic acid and were then stained routinely. The dopa reaction was not done, because dihydroxyphenylalanine was not available during the course of this study.

Summary of Cases

The cases will be listed in the order in which they were received in this laboratory for study and diagnosis.

Case 1 (MP-5760). A male Boston terrier, 12 years old, had a 30 by 13 by 8 mm., dark brown, gray mottled, firm, noncancerous melanoma, covered by ulcerated skin, in the right axilla.

Case 2 (46R-47). A female Chihuahua, 9 years old, had multiple cutaneous tumors at autopsy. Two submitted for study were noncancerous melanomas measuring 15 by 8 by 3.5 mm. and 25 by 15 by 1.5 mm.

Case 3 (46R-146). A male Airedale terrier, 14 years old, had many dark brown tumors on the tail. The one studied measured 17 by 13 by 12 mm. and was a firm, dark brown, skin-covered, noncancerous melanoma with a 2 mm. pedicle. Also present was a 26 by 17 by 13 mm., pink-gray, red mottled, ulcerated, cancerous melanoma of the right buccal region.

Case 4 (46R-156). A female Pekingese, 12 years old, presented a 10 mm., firm, gray, brown-streaked, noncancerous melanoma of a left eyelid.

* Drs. L. R. Phillips and B. S. Burkhardt of Lakewood, Colorado (10 cases), Dr. Rue Jensen of Colorado A. and M. College (9 cases), Drs. D. B. LyVere and L. S. Peavy of Denver (2 cases each), and Drs. M. H. Camner, F. T. Candlin, C. L. Davis, and J. R. Naylor of Denver, Dr. J. R. Durigg of Aurora, Colorado, Drs. G. H. Gilbert and V. D. Stauffer of Arvada, Colorado, Drs. J. C. Flint and R. A. Nipko of Salt Lake City, and Dr. W. J. Zontine of Lancaster, California (1 case each).

Case 5 (47R-5). A male German shepherd, 14 years old, had an 8 mm., firm, dark brown, noncancerous melanoma in the left metacarpal region.

Case 6 (47R-7). A female Boston terrier, 9 years old, presented a firm, black, skin-covered, noncancerous melanoma on the right hock, measuring 11 by 9 by 7 mm.

Case 7 (47R-174). On a male Scotch terrier, 14 years old, there were a firm, black, noncancerous melanoma of the left lower eyelid, which measured 18 by 8 by 4 mm., and an irregular, firm, black, cancerous melanoma in the right temporal region, measuring 20 by 17 by 9 mm.

Case 8 (47R-311). A male English setter, 14 years old, had a cancerous melanoma on the posterior wall of the pharynx, from which 32 gm. of tissue were submitted for examination. The dog was unable to swallow even liquids and was emaciated and dehydrated.

Case 9 (47R-314). A male Belgian shepherd, 11½ years old, presented two cancerous melanomas along the left costal margin. The first was 50 by 33 by 21 mm., firm, black and gray mottled, and had been present for 3 months. The second was 95 by 65 by 35 mm., pale yellow and gray, firm, divided into nodules 1 to 15 mm. in diameter, invaded the overlying skin, and had been present for 9 months.

Case 10 (47R-353). A female Airedale, 10 years old, had two noncancerous melanomas: one, 20 by 20 by 15 mm., pedunculated, covered by ulcerated skin, firm, gray and black mottled, was in the sternal region; the other, 50 by 35 by 30 mm., fairly soft to firm, and jet black, was in the left posterior phalangeal region.

Case 11 (47R-384). A male cocker spaniel, 7 years old, had had a firm, black, noncancerous melanoma, marked by a 2 mm. gray area, at the outer canthus of the left eye for 2 months. It measured 12 by 7 by 6 mm.

Case 12 (47R-487). A female mongrel Boston terrier, 10 years old, had had a 47 by 36 by 35 mm., firm, coarsely lobulated, gray and black mottled, cancerous melanoma covered by ulcerated skin for 3 months. Clinical evidence of metastases to the regional lymph nodes was observed 5 months after wide excision.

Case 13 (47R-518). A male Scotch terrier, 5 years old, presented a firm, gray, brown mottled, cancerous melanoma, measuring 15 by 10 by 10 mm., which had invaded the bone and marrow of a phalanx of the left forefoot.

Case 14 (47R-614). A male English springer spaniel, 13 years old, had an 18 mm., firm, gray, cancerous melanoma of the buccal region with metastases in the cerebral hemispheres. One metastasis was 9 mm. and dark brown; the other, 11 mm. and gray.

Case 15 (47R-646). A male English springer spaniel, 11 years old, had a cancerous melanoma, site unspecified, and metastases up to 2 cm. in diameter in the omentum, spleen, and lungs. Only the metastases were available for histologic examination.

Case 16 (47R-654). A female collie, 10 years old, had a cancerous melanoma of the nictitating membrane of one eye, measuring 10 by 10 by 5 mm.

Case 17 (47R-659). A male Irish water spaniel, 11 years old, had a 25 by 20 by 10 mm. cancerous melanoma, site unspecified.

Case 18 (47R-685). A male shepherd dog, 6 years old, presented a soft to firm, tan, dark red mottled, cancerous melanoma, measuring 32 by 25 by 24 mm., which had developed over the right costal margin during 6 months.

Case 19 (47R-690). A male cocker spaniel, 7 years old, had had a 12 by 9 by 5 mm. cancerous melanoma in the left gluteal region for 4 months.

Case 20 (47R-718). A spayed female Airedale, 12 years old, had a firm, dark brown, cancerous melanoma, weighing 935 gm., of the left hind leg. There were dark brown metastases in lymph nodes (left inguinal, 130 gm.; pelvic, 300 gm.; and thoracic, 330 gm.), heart, lungs, pancreas and kidneys, and, to a lesser degree, in the liver, ileum, and pleura. The brain contained no metastases. Four years before euthanasia, the primary neoplasm had been noted on the left hock and it gradually

spread to involve the posterolateral aspect of the tibial region and the distal third of the thigh. Clinically, emaciation and great enlargement of the inguinal lymph nodes were noted.

Case 21 (48R-28). A male English springer spaniel, 11 years old, had a cancerous melanoma, site unspecified, with multiple metastases in lungs and spleen. Only the metastases were available for histologic examination.

Case 22 (48R-31). A female Scotch terrier, 10 years old, presented a cancerous melanoma in the left thoracic region with metastases up to 2 cm. in diameter in the lungs, spleen, and kidneys. Only the metastases were available for histologic study.

Case 23 (48R-59). A female Scotch terrier, 9 years old, had had a spongy, pale yellow and dark brown mottled, pedunculated, ulcerated, noncancerous melanoma of the left thoracic region for 1 year. It measured 37 by 32 by 19 mm.

Case 24 (48R-130). A male Scotch terrier, 6 years old, had had a 10 by 4 by 2 mm., firm, gray-black, noncancerous melanoma of the left inner canthus for 6 months.

Case 25 (48R-162). A male cocker spaniel, 13 years old, had a firm, dark gray-brown, cancerous melanoma of the buccal region, which measured 25 by 22 by 15 mm.

Case 26 (48R-180). A male German shepherd, 10 years old, had a 6 by 5 by 4 mm. noncancerous melanoma of the right inner canthus.

Case 27 (48R-203). A female English springer spaniel, 4 years old, had a firm, black, noncancerous melanoma, measuring 8 by 7 by 5 mm., of the left lower eyelid next to the medial canthus.

Case 28 (48R-231). An old dog of unknown sex and breed had a 2 by 1 by 1 cm., firm, black, noncancerous melanoma of an eyelid.

Case 29 (48R-235). A female Scotch terrier, 3 years old, had a cancerous melanoma over the costal margin with metastases in the lungs and brain up to 1 cm. in diameter.

Case 30 (48R-251). A male Scotch terrier, 13 years old, had had for several months a firm, gray and brown mottled, cancerous melanoma, weighing 13 gm., of the left buccal region.

Case 31 (48R-267). A male Boston terrier, 14 years old, had a noncancerous melanoma on the tip of the right ear for more than a year. It measured 10 by 7 by 5 mm.

Analysis of Cases

Statistics on sex, age, and breed are based on the 30 dogs about which these data were known. Nineteen were males. In only 3 was the age less than 6 years, the remainder being 6 to 14 years old. The breeds represented included: terrier, 14 (Scotch, 7; Boston, 4; Airedale, 3), spaniel, 8 (English springer, 4; cocker, 3; Irish water, 1), shepherd, 4, and Chihuahua, Pekingese, English setter, and collie, 1 each. All of these breeds, with the exception of the Chihuahua, have heavily or moderately pigmented skin. The absence of the fox terrier from the dogs affected by melanomas is probably significant, since this breed, both smooth-coated and wire-haired, has relatively nonpigmented skin, but is often subject to other spontaneous neoplasms.[20] These data indicate that the breeds with more heavily pigmented skin are more susceptible to melanoma, as contrasted to humans, among whom those with lightly pigmented skin tend to be more often affected by melanoma. Of the 36 melanomas available for study, as primary growths or as metas-

tases, or as both, 17 were noncancerous and 19 were cancerous. Of these 36, 14 were located on the head, 8 on the thorax, 7 on the extremities, and 1 each on the neck and tail. The location of 5 tumors was not specified. Particularly notable was the predilection of the primary growth for the eyelids in 8 cases and for the cheeks in 4 cases. These 36 melanomas accounted for more than 5 per cent of the canine neoplasms studied in this laboratory.

Gross Findings

The dimensions or weight of 32 primary melanomas were available. By rough calculation, 15 of the 17 noncancerous tumors had a volume of 80 to 6000 cmm., and the 2 remaining, 22,496 and 52,500 cmm. Nine of 15 cancerous melanomas with available data had a volume of 8250 to 935,000 cmm. and the 6 remaining, 540 to 5832 cmm. These figures indicated that a given melanoma had a 70 per cent chance of being noncancerous if its volume were 6 cc. or smaller and an 80 per cent chance of being cancerous if its volume were 8 cc. or greater.

The primary neoplasms were covered by skin, which usually was heavily or moderately pigmented and often was ulcerated in small (Fig. 1) or large (Fig. 2) areas. Sometimes the tumor was pedunculated (Fig. 3). The cut section was spongy, fairly firm, and varied from relatively uniform dark brown (Fig. 1) to brown mottled with gray or yellow (Fig. 3), and sometimes even to jet black. Lobulation was fairly common in the larger tumors (Figs. 2 and 4), which were composed of dark brown, gray-brown, and gray nodules of varying size. An exceptionally large melanoma (Fig. 5), which produced many metastases (Fig. 6), was diffusely dark brown and, like other heavily pigmented growths (Fig. 1), left a slimy brown fluid on the knife on sectioning. Two or more primary melanomas were found in 4 dogs. At autopsy, metastases (Fig. 6) were observed in 6 dogs, 5 times in the lungs, 3 in the spleen, 2 in the kidneys, 2 in the brain, and once each in the lymph nodes, heart, ileum, liver, pancreas, omentum, and pleura. The metastases varied from 1 to 5 mm. up to 20 to 25 mm. in diameters.

Microscopic Examination

The noncancerous melanomas consisted of polyhedral cells (Fig. 7), of stubby or long spindle cells (Fig. 8), or of tapered slender cells. Also quite frequent were cells with multiple, thin, dendritic processes. The polyhedral cells tended to be grouped in solid masses, in small alveolar patterns, or in larger compartments. The long spindle cells and the tapered slender cells were usually arranged in sweeping bundles, were

sometimes mingled with small nerve trunks in the subcutaneous tissue, and resembled proliferated nerve sheath cells, especially in areas where some nuclear palisading was noted. As a rule, the cells of noncancerous melanomas were set together in rather close-knit fashion (Figs. 9 and 10), except in areas of edema or near areas of ulceration, in which they were spread apart. Whorls of cells were fairly frequent (Fig. 10) and recalled the structure of Meissner's corpuscles. The amount of acidophilic cytoplasm varied, but was notably conspicuous in the polyhedral forms. The ratio between cytoplasmic and nuclear volume was fairly constant, considering the shapes of the cells. Melanin pigment was scanty or absent and the nuclei were easily seen (Figs. 9 and 10) or was so abundant that the nuclei of the cells were practically obscured (Figs. 7 and 8), so that bleaching was necessary in order to see the nuclei. The cells with several thin, dendritic processes were usually heavily laden with melanin granules. The nuclei were almost invariably single, were round or oval in the polyhedral cells, and were oval to oblong in the long spindle and slender tapered cells. Nuclei of a given shape were quite close to each other in size. The chromatin net (Figs. 9 and 10) was fine and homogeneous, was distributed evenly in the nucleus, and filled the entire nucleus, which usually had a sharply demarcated border and contained a definite, nearly always single, small nucleolus. Occasional or scattered, regular mitotic figures were noted, especially when the cells were near areas of inflammation at the surface of an ulcerated neoplasm. The overlying epidermis, when intact and when distant from the denuded areas, revealed granules of melanin pigment, moderate to heavy in the bottom row of epidermal cells and in nearly all of the cells of the epidermis in some. This epidermal pigmentation was noted also in the cancerous melanomas.

The shape of the cells in the cancerous melanomas was similar to that of the cells in the noncancerous tumors, but polyhedral and stubby spindle cells predominated (Figs. 11 and 12). Forms with dendritic processes were rare or absent. In areas free of infection or edema the cells tended to have a much looser arrangement. They usually were massed, alveolar, or in compartments, or, less commonly, in short bundles. The tendency to resemble nerve sheath tumors in some areas was not a feature of the cancerous melanomas. The cells, as a rule, were larger and had relatively less acidophilic cytoplasm, which in some areas of a few neoplasms tended to be clear and to contain pyknotic nuclei. The nuclei were much larger in relation to the volume of the cytoplasm than in the noncancerous melanomas. The amount of melanin in the cells was of little aid in the differentiation of noncancerous from can-

cerous melanomas, although the cells of the cancers with the more anaplastic characteristics (Fig. 12) tended to inclose little or no melanin. In most of the others (Fig. 11), the melanin varied from slight to abundant, but was hardly ever so plentiful as to obscure the nuclei. The nuclei usually were round or oval and plump, and varied widely in size, some being giant and bizarre (Figs. 13 and 14). The nuclear chromatin was coarse, unevenly distributed, and tended to occupy a peripheral position in the nucleus and to cause an apparent thickening of the perinuclear membrane by its margination. Nucleoli were enlarged and prominent; sometimes 2 or 3 were present. Deep invasion of the subcutaneous adipose tissue, of skeletal muscle, or lymphatics, and of veins was noted with several cancerous melanomas. Areas of necrosis and hemorrhage were fairly common. The microscopic features of the metastases were similar to those of the primary neoplasms, when both were available for study.

Summary

Of 36 melanomas observed in 31 dogs, 17 were noncancerous and 19 were cancerous.

In the 30 dogs in which sex and age were known, 19 were males. Only 3 were less than 6 years old, the remainder being 6 to 14 years.

The breeds chiefly affected were those with heavily or moderately pigmented skin.

Melanomas of the head, thorax, and extremities accounted for 29 of the neoplasms observed. The predilection of the primary growth for eyelids (8 cases) and for cheeks (4 cases) was especially noteworthy. Four dogs had two or more primary melanomas. Six dogs showed metastases at autopsy, the lungs being affected in 5.

The melanomas with a volume of 8 cc. or more had about an 80 per cent chance of being *cancerous*. The melanomas with a volume of 6 cc. or less had about a 70 per cent chance of being *noncancerous*.

The looseness of cell arrangement, the preponderance of polyhedral and stubby spindle cells, the large size of nuclei in relation to cell volume, the increased number of round and oval nuclei, the variation in size of nuclei, multiplicity of nuclei, coarsening and margination of chromatin, and increase in size and number of nucleoli were all of considerable aid in differentiating cancerous from noncancerous melanoma. In this differentiation, mitotic figures were of some help, if they were numerous and irregular or pluripolar. The amount of melanin in the neoplastic cells varied in different areas of both noncancerous and cancerous melanoma. A tendency for lessened melanin production was observed in the more anaplastic cells of a given cancerous melanoma. Local invasion

of adipose tissue, skeletal muscle, lymphatics, and veins, and metastasis afforded ultimate evidence that a melanoma was cancerous.

It is considered that good reasons exist for designating the innocent neoplasm of melanin-forming cells as "noncancerous melanoma" and for the application of the name "cancerous melanoma" to the anaplastic, invasive, metastasizing neoplasm of melanin-forming cells.

Mr. Glenn E. Mills gave technical assistance with photography.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

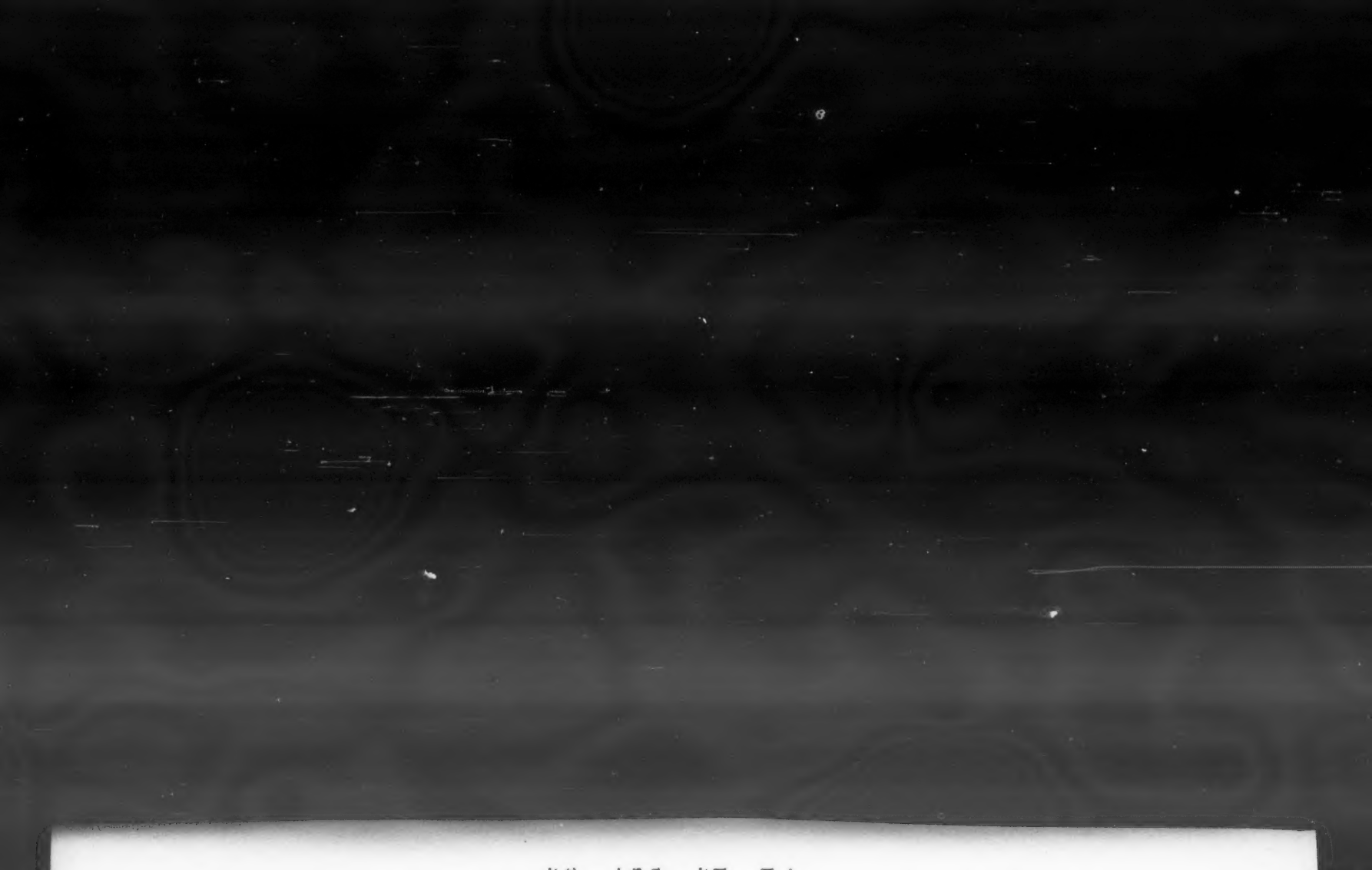
PLATE 44

FIG. 1. Case 10. Noncancerous melanoma. The skin surface is shown in the upper figure. Lighter areas are locally ulcerated. The homogeneous, dark brown cut surface is illustrated below.

FIG. 2. Case 12. Cancerous melanoma. The skin surface in the upper figure is ulcerated over its superior portion; and the line of excision of the broad base appears in the inferior portion. The nodular, brown, gray mottled cut surface is shown in the lower figure.

FIG. 3. Case 23. Noncancerous melanoma. Skin surfaces are shown in the upper and lower figures. The pedicle appears in the upper illustration. The brown and pale yellow mottled cut surface is shown in the middle figure.

FIG. 4. Case 9. Two cancerous melanomas with nodular, brown and gray mottled cut sections. Skeletal muscle is invaded at the lower edge of the larger neoplasm.



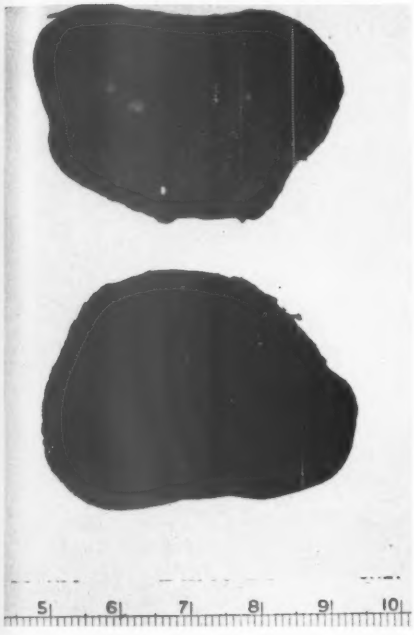


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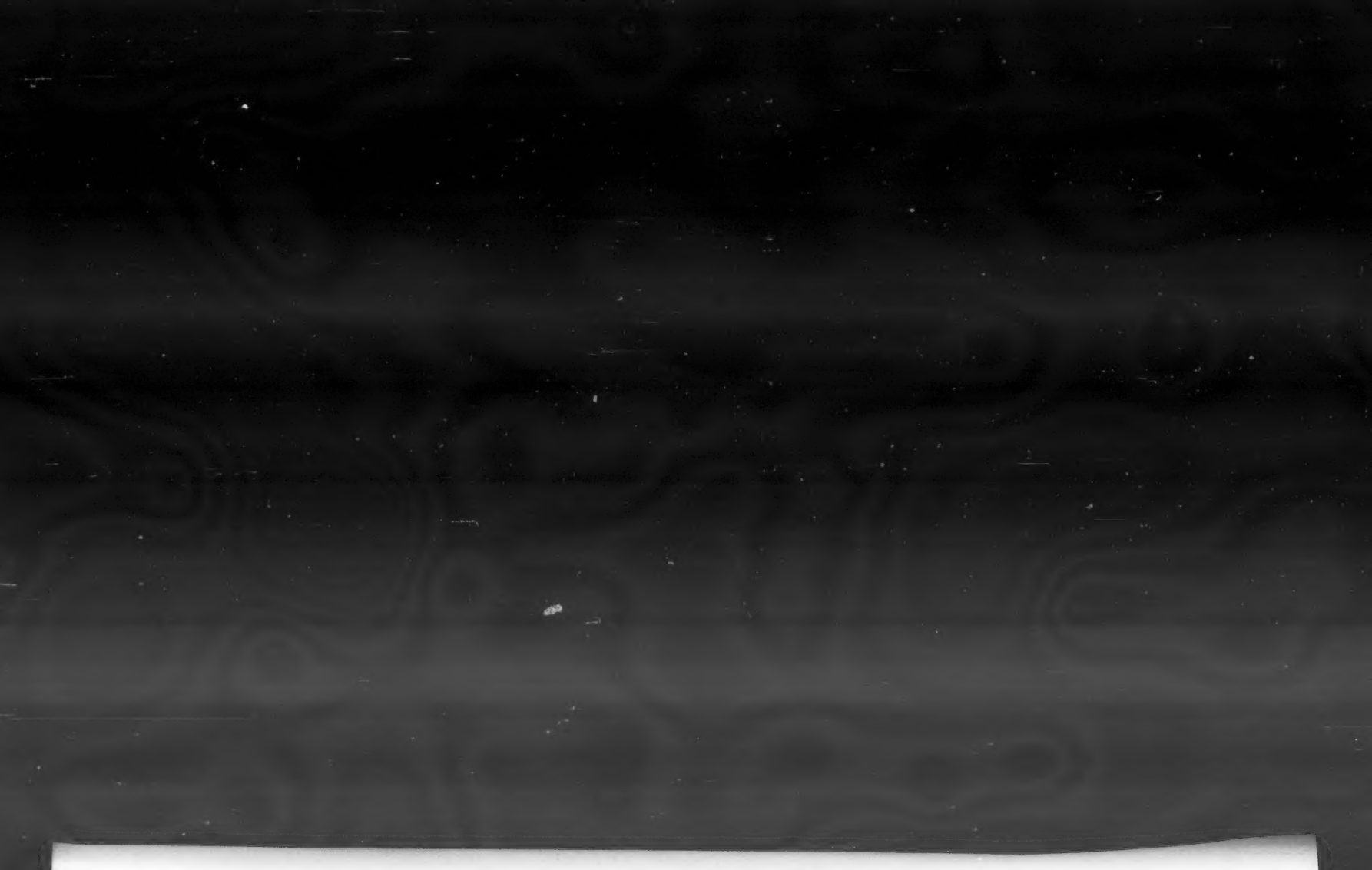
PLATE 45

FIG. 5. Case 20. Cancerous melanoma, lateral aspect, with a homogeneous, deep brown surface after sectioning. The lower skin-covered border was in the region of the hock and the upper fascia-covered border in the region of the thigh.

FIG. 6. Case 20. Metastases of cancerous melanoma in lung and kidney. Metastases in thoracic and inguinal lymph nodes and in pancreas are shown also (viewed from left to right in the lower part of the picture).

FIG. 7. Case 5. Noncancerous melanoma. Melanin is present in the cells of all layers of the epidermis at the left. Masses of melanin-laden, polyhedral tumor cells in the dermis and upper hypodermis. Hematoxylin and eosin stain. × 150.

FIG. 8. Case 6. Noncancerous melanoma. In a rete peg of hypertrophied epidermis in the lower left-hand corner, the epithelial cells contain much melanin. Melanin-laden, long spindle-shaped tumor cells are abundant in the dermis and upper hypodermis. Hematoxylin and eosin stain. × 150.



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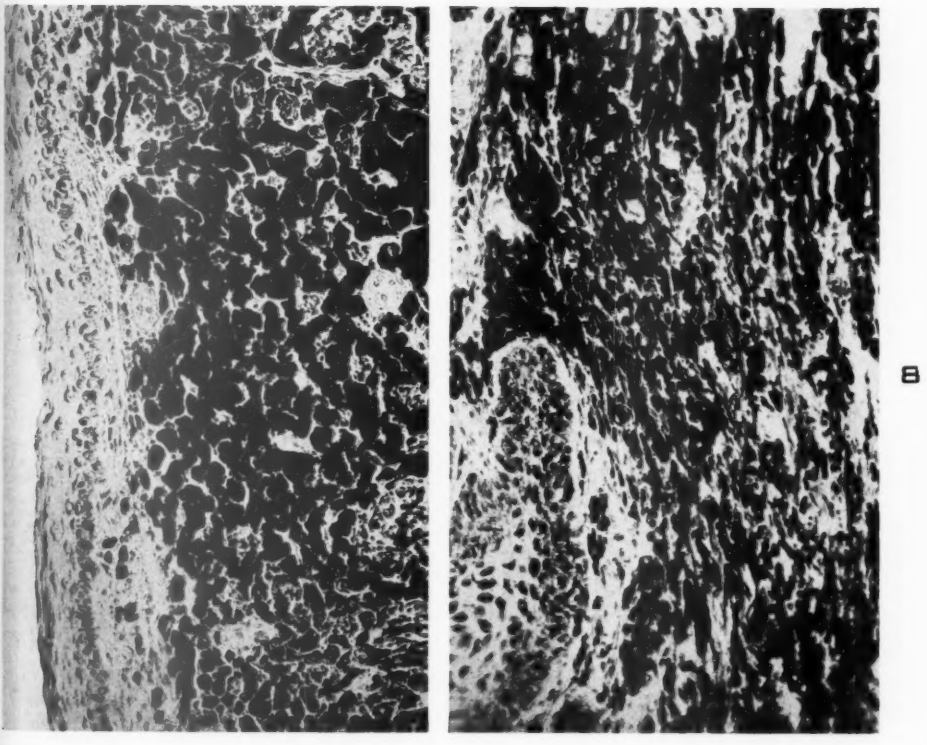

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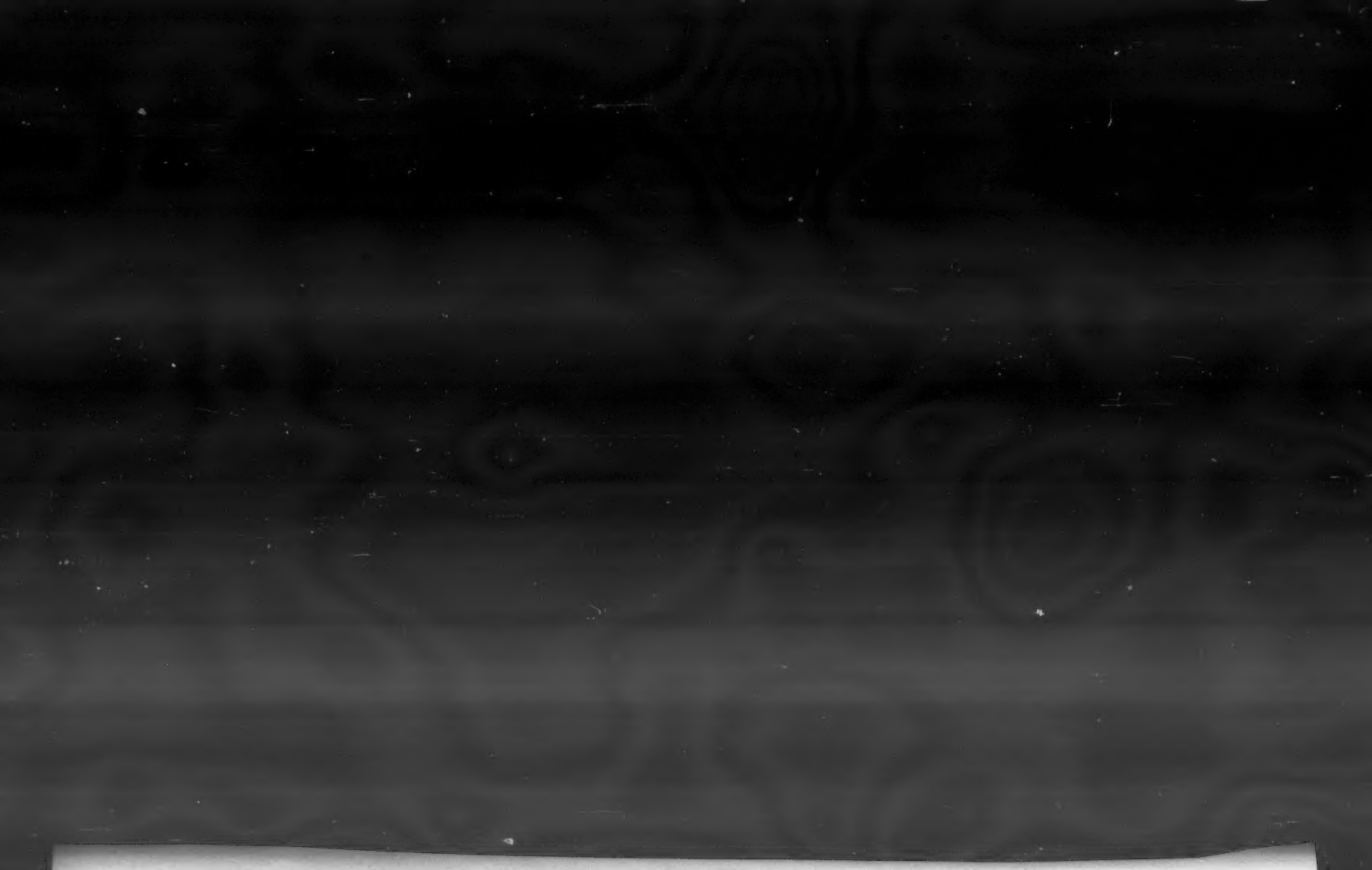
PLATE 46

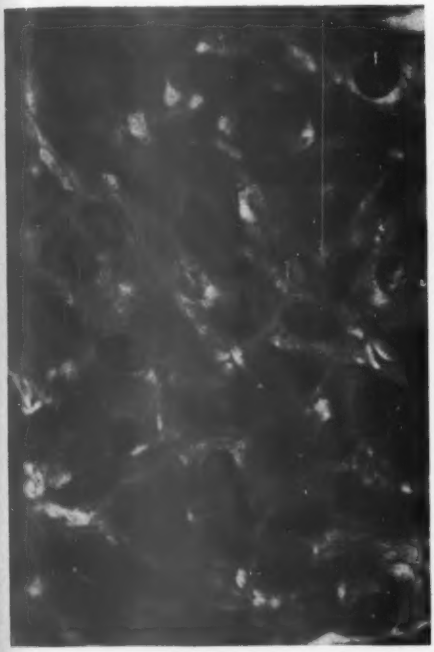
FIG. 9. Case 11. Noncancerous melanoma with compactly arranged polyhedral and stubby spindle-shaped cells containing no melanin. The nuclei are round or oval. The chromatin net is regular, fine, and homogeneous. Nucleoli are small and discrete. Hematoxylin and eosin stain. × 900.

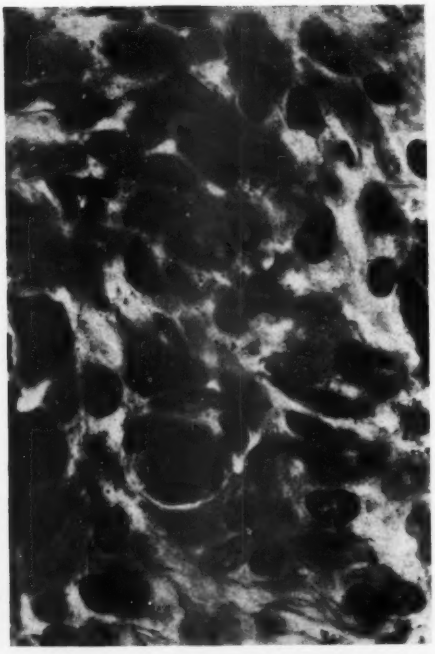
FIG. 10. Case 11. Noncancerous melanoma. Stubby and long spindle-shaped cells predominate in this field. Melanin is moderate to abundant. The whorled arrangement just below and to the left of the center suggests a Meissner's corpuscle. There are fibrillar cellular processes in the lower portion of the field. Hematoxylin and eosin stain. × 900.

FIG. 11. Case 7. Cancerous melanoma showing a loose arrangement of stubby spindle-shaped and polyhedral cells. Melanin content and nuclear sizes are variable. The chromatin is coarse and irregular and the nucleoli are enlarged and prominent. Hematoxylin and eosin stain. × 450.

FIG. 12. Case 3. Cancerous melanoma with stubby spindle-shaped cells loosely arranged and containing no melanin. The nuclei are plump and the chromatin, coarse and uneven. Nucleoli are enlarged. Hematoxylin and eosin stain. × 450.







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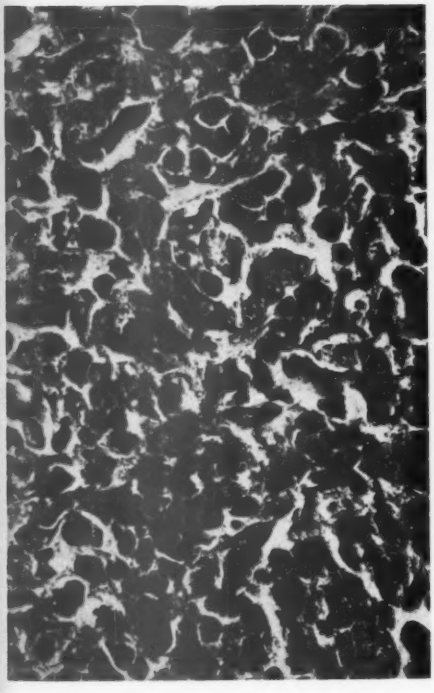

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PLATE 47

FIG. 13. Case 7. Cancerous melanoma showing a loose arrangement of stubby spindle-shaped and polyhedral cells. Melanin content and nuclear sizes are variable. The chromatin is coarse and irregular and the nucleoli are enlarged and prominent. Hematoxylin and eosin stain. × 900.

FIG. 14. Case 3. Cancerous melanoma with stubby spindle-shaped cells loosely arranged and containing no melanin. The nuclei are plump and the chromatin, coarse and uneven. Nucleoli are enlarged. Hematoxylin and eosin stain. × 900.

FIG. 15. Case 3. Cancerous melanoma with stubby spindle-shaped cells loosely arranged and containing no melanin. The nuclei are plump and the chromatin, coarse and uneven. Nucleoli are enlarged. Of note is the plump mitotic figure just below the right of the center. Hematoxylin and eosin stain. × 900.

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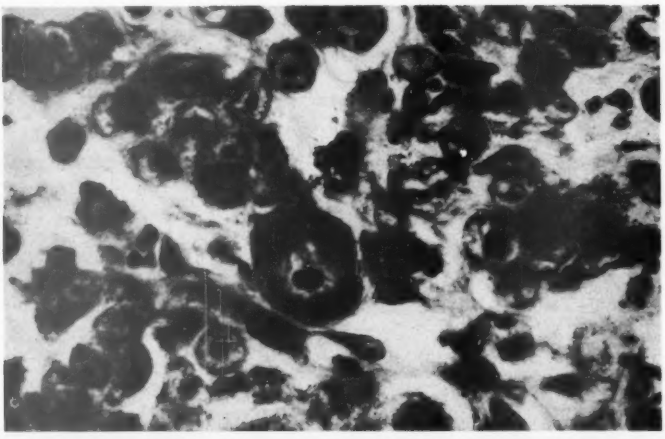

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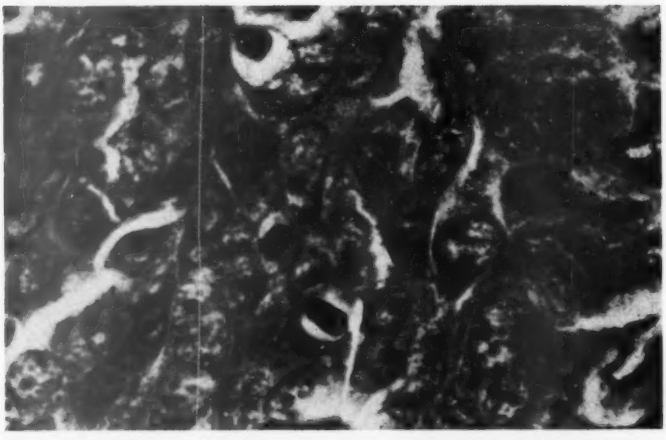

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